Microfluidic approaches for cell-based molecular diagnosis

Dong Jun Lee,¹ John Mai,² and Tony Jun Huang^{1,3,a)}

¹*Department of Biomedical Engineering, Duke University, Durham, North Carolina 27708, USA*

²*Alfred E. Mann Institute for Biomedical Engineering, University of Southern California, Los Angeles, California 90089, USA*

³*Department of Mechanical Engineering and Material Science, Duke University, Durham, North Carolina 27708, USA*

(Received 26 March 2018; accepted 27 August 2018; published online 14 September 2018)

The search for next-generation biomarkers has enabled cell-based diagnostics in a number of disciplines ranging from oncology to pharmacogenetics. However, cell-based diagnostics are still far from clinical reality due to the complex assays and associated protocols which typically require cell isolation, lysis, DNA extraction, amplification, and detection steps. Leveraging recent advances in microfluidics, many biochemical assays have been translated onto microfluidic platforms. We have compared and summarized recent advances in modular approaches toward the realization of fully-integrated, cell-based molecular diagnostics for clinical and point-of-care applications. *Published by AIP Publishing.* <https://doi.org/10.1063/1.5030891>

I. INTRODUCTION

Over the past few decades, a number of biochemical protocols, which have been traditionally conducted using bulk sample volumes, have been implemented on microfluidic platforms.^{1–5} Typical microfluidic devices have dimensions ranging from 1 to 500 μm , which are in the same scale as those of cells and other biologically relevant samples. This Reynolds number range also dramatically changes from turbulent to laminar flow regimes.⁶ This allows for more precise manipulation of the fluids and bioparticles within these fluids and hence makes it ideal for diagnostic applications. Also, it requires less sample volume and allows for the sequential integration of assay steps in an automated manner.⁷ Most of these initial efforts have been focused on the implementation of simple biochemical assays based on enzyme-linked immunosorbent assay (ELISA) which detects a single or a set of protein-based biomarkers for a specific disease.⁸ As the designs of microfluidic devices advanced, the complexity of an assay that can be performed on a microfluidic platform rapidly increased. It is now possible to perform complex biochemical assays such as polymerase chain reaction (PCR),⁹ DNA/RNA sequencing,¹⁰ fluorescence *in situ* hybridization (FISH),¹¹ and even the systematic enrichment of ligands by exponential enrichment (SELEX).^{12,13} These advancements bring microfluidic technology into the field of molecular diagnostics, which provides a better understanding of the underlying genetic mechanisms than phenotypic approaches. However, while these approaches can directly be applied to the detection of protein-/DNA-based biomarkers carried in biological fluids, there is still a significant body of diagnostic protocols requiring the examination of genetic information hidden inside the cells.

Using cancer diagnostics as an example,¹⁴ clinical evidence shows that the role in which early diagnosis plays in improving a patient's long-term survival rate is as vital as selecting the appropriate treatments.¹⁵ For example, early detection of breast, colon, and rectum cancers has significantly reduced mortality.^{16,17} This is further supported by the 2010 National Cancer Institute vision document, which emphasizes a shift from therapeutic to preventive modalities.¹⁸ However, cancer diagnostics suffer from a multitude of problems. Cancer types are not identical between patients, and therefore, genotyping is critical for choosing an effective treatment strategy.¹⁹ Conventionally,

^{a)} Author to whom correspondence should be addressed: tony.huang@duke.edu

cancerous tissues are withdrawn from a patient by either a solid or liquid biopsy followed by extensive analytic procedures for cancer typing.^{20,21} However, such procedures are time-consuming, expensive, and are usually performed by a skilled clinical professional.

Another example is found in pharmacogenetics.^{22–24} It is now understood that the variability in the responses between different patients toward a drug may be attributed to the genetic variation due to single nucleotide polymorphism (SNP) which in turn emphasizes the need for new tools to enable personalized medicine.²² For various diseases ranging from AIDS to rheumatoid arthritis, the number of common genetic variations responsible for hypersensitivity toward drugs such as Abacavir,²² Azathioprine,²⁵ and Warfarin²⁶ has been identified. It is anticipated that genetic testing against these variations prior to treatment would help to select the most effective drugs while also minimizing any adverse drug reactions.

The aforementioned diagnostic procedures require direct examination of cells which entails more complicated sample preparation steps such as cell extraction, enrichment, lysis, and DNA extraction. Microfluidic-based devices are capable of efficiently performing every step of these sample preparation steps and protocols. This review summarizes the current status of cell-based molecular analysis using various microfluidic techniques. The general workflow for molecular analysis of cells is divided into four steps, namely, (1) cell separation, (2) cell lysis, (3) DNA extraction and amplification, and (4) biomarker detection. We will outline the microfluidic-based techniques used to perform each of these steps, as well as highlight various efforts to integrate them into micro-total-analysis-systems (μ TAS).

II. CELL SEPARATION

Normally, a heterogeneous suspension of a biological sample must be purified and enriched in order to collect a well-defined population of target cells while minimizing contamination from the presence of other cell types. Currently, methods for cell separation rely on either their physical properties, such as density, size, and dielectric properties, or affinity toward surface antigens present on the target cells.²⁷ An assessment of these techniques can be categorized by the following two major considerations:²⁸ (1) the resulting purity of the target sample and (2) the yield of the target sample.

Purity describes the percentage of target cells within a separated cell population. Obtaining a highly purified population of target cells has the potential to improve the confidence in the subsequent analysis and diagnostic decisions. However, it should be noted that the term “purity” can be misleading. For example, the separation of T-cells often relies on selection against CD4. However, there are a number of different sub-types of T cells which express CD4 such as Th1 and Th2 helper cells, memory T cells, naïve T cells, and effector T cells.²⁹ Therefore, separation based on characteristics shared between different types of cells could still result in a heterogeneous mixture which would influence the diagnosis results.

Yield refers to the ratio between the total number of cells sorted versus the total number of cells that were present in the original sample. One should be cautious in choosing a separation strategy because the yield primarily depends on the selected characteristic used for sorting. For example, circulating tumor cells (CTCs) are often selectively captured by using antibodies against epithelial cell adhesion molecules (EpCAMs). However, it has been suggested that the loss of EpCAM expression occurs after the epithelial to mesenchymal transition, giving rise to a cell population of EpCAM-negative CTCs.^{30,31} Therefore, suitable markers should be chosen to guarantee a high yield.

Generally, cell capture and separation processes can be categorized into their use of either positive or negative selection criteria.³² In positive selection, the cells of interest are selected via their affinity or conformity to criteria. However, in negative selection, unwanted cells are removed from the sample solution thus leaving behind only the cells of interest. The positive selection strategy can result in high purity (>99%) due to a highly specific ligand affinity; yet, the recovery ratio is often low. On the other hand, a negative selection strategy allows separation of the target cells without the need for a target-cell-labeling step, which could alter the physiological state of the target cells. Also, it can be applied to cells without specific markers. However, a negative selection

strategy often suffers from the inefficient removal of unwanted cells which reduces the purity of the final cell suspension.³²

A. Conventional cell separation techniques

The most common traditional technique for cell separation is via density gradient centrifugation.^{33,34} During centrifugation, cells immersed in a viscous medium experience different accelerations depending on their density and, as a result, separate into different cell types. Density gradient centrifugation can be seen as a bulk method due to its ability to separate a large number of cells, making it particularly applicable for cell separation in a blood sample, which contains $\sim 10^9$ cells/ml. However, it suffers from poor yield. Also, extreme shear forces during centrifugation exert physiological stress on the target cell population which could alter the subsequent expression profile.

An immunomagnetic-based approach is another popular conventional cell separation technique due to its ease of use and the minimal level of external forces exerted on the cell.³² Superparamagnetic microbeads are labeled with an antibody that is either specific to the target for positive selection or the unwanted cells for negative selection. The cell suspension is then incubated with these antibody-labeled magnetic microbeads for immunorecognition, and then an external magnetic field is applied to attract the microbead-cell complexes, resulting in a target-enriched supernatant or suspension, respectively, depending on the positive or negative selection criteria. This method has already been commercialized and the CellSearch system,³⁵ originally developed by Veridex LLC, has already been approved by the FDA for the separation of CTCs from patient blood samples.³⁶

Fluorescence-activated cell sorting (FACS) has been regarded as a gold standard for cell sorting, primarily due to its high purity, multi-parametric nature, and single-cell level sensitivity (Table I).³⁷ In FACS, one or more sets of antibodies are labeled with different fluorescence dyes which then specifically bind to surface markers present on the target cells. Hence, it is regarded as a positive separation technique. These labeled cells are then aligned and flow into a single stream of small droplets so that every cell is sequentially illuminated by a laser beam.³⁸ The light scattered by each cell is recorded simultaneously at different angles. The forward-scattering light is recorded by a detector on the opposite side of the laser beam and is correlated with the cell size. The side scattering light is recorded by a series of detectors positioned at a 90° angle to the beam axis. These detectors are equipped with different optical filters to record the fluorescence profile of the cell.³⁹ Finally, a downstream deflector element sorts the cell-containing droplets into separate collection tubes using a tangential electric field. FACS allows high-purity (>99%) cell separation based on multi-parametric selection criteria and can have a throughput as high as 100,000 cells/s (Table I).^{27,40} However, FACS suffers from drawbacks such as high cost, complexity, and limited biosafety and effects the viability of the sorted cells.

B. Microfluidic cell separation techniques

In the past decade, there has been an intensive effort to develop microfluidic platforms for cell separation due to the following anticipated benefits:²⁷

- (1) Trapping cells within a narrow fluidic streamline allows for precise cell manipulation.
- (2) The flow field generates an appropriate velocity gradient with dimensional scales comparable to the size of cells, which allows for cell sorting using hydrodynamic forces.
- (3) The microscale dimension enables concentration of electromagnetic fields and acoustic fields.
- (4) Scalable microfabrication techniques allow for the miniaturization of instrumentation, reduced cost, and simple integration with other microfluidic elements for downstream analysis.
- (5) The operation of microfluidic devices can be pre-programmed and automated to eliminate the need for skilled or trained operators.
- (6) The possibility of massively-parallel processing allows for high-throughput cell separation.

TABLE I. Summary of various conventional and microfluidic techniques for cell separation, cell lysis, DNA extraction and amplification, and biomarker detection.

Cell separation techniques			
Techniques	Throughput	Separation efficiency	Reference
FACS	$\sim 120 \mu\text{l}/\text{min}$	95%–100%	40
CellSearch	5 ml/h	91%–95%	35,128
Inertial flow	103 $\mu\text{l}/\text{min}$	99.10%	48
	800 $\mu\text{l}/\text{min}$	83%	49
Dielectrophoresis	60 $\mu\text{l}/\text{min}$	96%	52
	1.6 $\mu\text{l}/\text{min}$	96%	53
	315 $\mu\text{l}/\text{min}$	...	55
Acoustofluidics	2 $\mu\text{l}/\text{min}$	84%	56
	20 $\mu\text{l}/\text{min}$	90%	43
	10 ml/min	85%	61
Lysis techniques			
Techniques	Throughput	Lysis efficiency	Reference
Mechanical lysis	300 $\mu\text{l}/\text{min}$	99%	70
	5–7 min per operation	65% of the conventional methods	73
	3 min for 1 ml	...	74
Electrical lysis	...	93%–99%	79
	...	30%	82
	3 min per operation	...	78
Thermal lysis	2 min for 25 μl	...	84
	8 s for 75 μl	...	85
	32 min for 3.5 μl	...	86
Extraction techniques			
Techniques	Throughput	Extraction efficiency	Reference
Static SPE	2.6–14.2 ml/min	70% of the conventional methods	90
	4.16 $\mu\text{l}/\text{min}$	79% of loaded DNAs	91
	1.6 $\mu\text{l}/\text{min}$	48% of loaded DNAs	92
Dynamic SPE	7.0 $\mu\text{l}/\text{min}$	Similar to the conventional method	88
	45.0 $\mu\text{l}/\text{min}$	147% of the conventional method	93
Amplification techniques			
Techniques	Throughput	Thermocycling time	Reference
Microchamber PCR	...	3 min/cycle	94
Continuous PCR	0.34–4.37 $\mu\text{l}/\text{min}$	4.5–56.4 s/cycle	96
	0.09–0.12 $\mu\text{l}/\text{min}$	6 min/cycle	97
	1.8–8 $\mu\text{l}/\text{min}$	10–20 s/cycle	98
Droplet PCR	2.7 $\mu\text{l}/\text{min}$	29 s/cycle	100
Detection techniques			
Techniques	Detection time	Detection limit	Reference
Capillary electrophoresis	7 min	12.6–13.4 pM	104
Electrochemical detection	45–90 min	5 pg/ml	107
	90 min	2 aM	108
	20 min	1 pM	109
Microarray	15 min	10 nM	113
	<5 min	50 aM	114

FIG. 1. Schematic illustration of passive and active separation. Passive separation is a deterministic process where the sorting of cells is predominantly determined by the channel geometry, whereas active separation involves applying an external field force that could be dynamically controlled to manipulate the cells. (a) Inertial flow-based separation. A wall effect based lift force pushes cells toward the centerline of the channel while a shear gradient lift force pushes cells away from the centerline of the channel. These two opposing lift forces establish an equilibrium position on the lateral axis of the channel. The magnitude of each force is directly proportional to the size of the cell, and therefore cells with different sizes become spatially separated after traveling a given distance.⁴¹ Reprinted with permission from C. Liu and G. Hu, *Micromachines* **8**(3), 73 (2017). Copyright 2017 Multidisciplinary Digital Publishing Institute.⁴¹ (b) Dielectrophoretic separation. Polarizable particles, such as cells, experience a net force when exposed to a non-uniform electric field and are therefore attracted to or repelled from the external electric field.⁴² Reprinted with permission from D. R. Gossett *et al.*, *Anal. Bioanal. Chem.* **397**(8), 3249–3267 (2010). Copyright 2010 Springer Science+Business Media.⁴² (c) Acoustofluidic separation. Larger cells experience a larger acoustic radiation force (F_{rl}) than smaller cells (F_{rs}). As a result, larger cells have a larger vertical displacement (normal to the flow direction) than smaller cells. F_{dl} and F_{dr} are the drag force experienced by larger cells and smaller cells, respectively.⁴³ Reprinted with permission from P. Li *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **112**(16), 4970–4975 (2015). Copyright 2015 The National Academies of Sciences, Engineering, and Medicine.⁴³

Generally, the microfluidic-based cell sorting strategies that have been developed over the past decade could be categorized into passive and active separation (Fig. 1).^{27,41–43} Passive separation is a deterministic process where the sorting of cells is predominantly determined by the channel geometry which intrinsically shapes the hydrodynamic forces, whereas active separation involves applying an external force that can be dynamically controlled to manipulate the cells.

One example of a passive separation method utilizes the interaction between inertial and viscous forces, namely, the shear-stress-induced lift force and the wall-induced lift force.⁴⁴ The shear-induced lift force arises due to the difference in fluid velocities along the cross-sectional axis of a microchannel, and it effectively pushes larger cells further away from the centerline of the channel. The wall-induced lift forces are generated when the immersed cells are flowing near the wall and effectively repels larger cells further away from the wall [Fig. 1(a)].^{41,45,46} These two opposing lift forces establish an equilibrium position along the lateral axis of the channel, and because the magnitude of each force is directly proportional to the size of the cell, cells with different sizes become spatially separated after traveling a given distance. Equation (1) describes the net inertial force experienced by a small rigid particle in a Poiseuille flow where F_L , ρ , U , a , and H are the non-dimensional lift coefficient, fluid density, average velocity, diameter of a particle, and characteristic channel dimension, respectively.⁴⁵

$$F_L = f_L \rho U^2 a^4 / H^2. \quad (1)$$

Initially, inertial-flow-based techniques utilized a simple curved geometry to separate cells.⁴⁷ However, these techniques suffer from low throughput and purity because although the different sized particles experience different inertial lift forces, their equilibrium position is about the same after traveling a sufficient distance down the channel. Therefore, there have been a number of

FIG. 2. Microfluidic-based cell separation. (a) A series of contraction-expansions in a microchannel device that utilizes the balance between the inertial lift force and Dean drag force for circulating tumor cell (CTC) separation.⁴⁸ Reprinted with permission from M. G. Lee *et al.*, *Anal. Chem.* **85**(13), 6213–6218 (2013). Copyright 2013 American Chemical Society Publications.⁴⁸ (b) A dielectrophoresis device utilizing wireless bipolar electrodes for single CTC separation. The wireless electrodes are embedded inside the channel and communicate with an external electrode through inductance.⁵⁵ Reprinted with permission from M. Li and R. K. Anand, *J. Am. Chem. Soc.* **139**(26), 8950–8959 (2017). Copyright 2017 American Chemical Society Publications.⁵⁵ (c) An acoustofluidic fluorescent activated cell sorter (FACS) device. Interdigital transducers (IDTs) generate short bursts of standing surface acoustic waves (SSAWs) to deflect cells to different outlets.⁵⁶ Reprinted with permission from A. A. Nawaz *et al.*, *Anal. Chem.* **87**(24), 12051–12058 (2015). Copyright 2015 American Chemical Society Publications.⁵⁶

efforts to establish distinct equilibrium positions for particles of different sizes. For example, the inertial lift force has been coupled with the Dean drag force to separate target cells, using a series of connected sections of expanding and contracting microfluidic channels [Fig. 2(a)].⁴⁸ The balance between these two forces increased the lateral separation for various cancer cells (MCF-7, SK-BR-3, and HCC70) spiked in whole human blood with a yield of 99.1% at a throughput of 103 $\mu\text{l}/\text{min}$ (Table I). Similarly, CTCs from lung and breast cancer patients have been efficiently isolated utilizing a parallel array of rectangular reservoirs to generate micro-vortices which selectively capture and separate the larger CTCs from other blood components at a throughput of 800 $\mu\text{l}/\text{min}$ whole blood (Table I).⁴⁹ However, inertial-flow-based techniques suffer from poor purity because of their limited ability to separate target CTCs from white blood cells due to their similar size ranges.

Dielectrophoresis (DEP) refers to a phenomenon where polarizable particles, such as cells, experience a net force when exposed to a non-uniform electric field [Fig. 1(b)].^{42,50} Equation (2) describes the net force experienced by the cell where r , ϵ_m , and E are the radius of the cell, the permittivity of the surrounding medium, and the electric field, respectively. The $f_{CM}(\omega)$ is the Clausius-Mossotti factor which depends on both the permittivities of the cell and the surrounding medium.⁵¹

$$F_{DEP} = 2\pi r \epsilon_m \text{Re}[f_{CM}(\omega)] \nabla E^2. \quad (2)$$

Although the direction of the DEP force is determined by the gradient of the square of the electric field, the Clausius-Mossotti factor determines the sign and hence the direction of movement. Therefore, depending on the relative permittivity difference between the cells and the surrounding medium, the cells can be focused toward or away from the electrodes.⁴²

The major advantage of active separation techniques such as DEP is their ability to precisely manipulate specific cells as well as dynamic force control in real time. This has been demonstrated by a DEP-based fluorescence activated droplet sorter (DEP-FADS).⁵² Here, a droplet containing *E. coli* expressing a specific reporter gene has been selectively identified through a fluorescence

signal and then sorted using electrophoretic actuation with a throughput of 60 $\mu\text{l}/\text{min}$ (Table I). Also, DEP techniques allow for separation based on both cell size and dielectric properties of each cell type, which results in a higher selectivity over inertial-flow-based techniques that solely rely on the size of the cell. For example, DEP-based cell separation utilizing interdigitated comb-like electrodes have demonstrated 96% capture efficiency for MDA231 breast cancer cells from normal blood cells (Table I).⁵³ However, it should be noted that DEP-based methods suffer from a relatively low throughput (0.20–20 $\mu\text{l}/\text{min}$) compared to inertial-flow-based methods (200–2000 $\mu\text{l}/\text{min}$) due to the inherent short-range effects of the electric field gradient and design configurations.⁵⁴ Recently, Li and Anand have reported a DEP-based cell separation device using a wireless bipolar electrode array [Fig. 2(b)].⁵⁵ Here, wireless electrodes are directly embedded inside the channel and communicate with an external electrode through inductance. This unique design allows for a significant reduction in spacing between the electrode and sample solution. This allows for a higher electric field gradient and results in a much higher throughput (315 $\mu\text{l}/\text{min}$) (Table I).

Acoustofluidics (i.e., the fusion of acoustics and microfluidics) offers another type of active cell separation approach [Fig. 1(c)].^{43,56–64} Typically, two opposing transducers will each generate acoustic waves which establish a standing acoustic field in a given microfluidic volume. Any immersed particles within this fluctuating fluidic medium experience a radiation force ($F_{\text{radiation}}$) which focuses on the particles toward a pressure node or an antinode depending on the acoustic contrast factors (Φ). $F_{\text{radiation}}$ and Φ are determined by Eqs. (3)⁴ and (4),⁶⁵ respectively, as follows:

$$F_{\text{radiation}} = -\left(\frac{\pi P_0^2 V_p \beta_m}{2\lambda}\right) \Phi \sin \frac{4\pi x}{\lambda}, \quad (3)$$

$$\Phi = \frac{5\rho_p - 2\rho_m}{2\rho_p + \rho_m} - \frac{\beta_p}{\beta_m}. \quad (4)$$

Here, P_0 and V_p denote the acoustic pressure and the volume of the particle, respectively; ρ_m and ρ_p are the density of the medium and particles, respectively; and β_m and β_p are the compressibilities of the medium and particles, respectively. Since the flow in the microfluidic channels is laminar, particles with different physical properties can be continuously separated.

Separation of cultured cancer cells has recently been demonstrated by Ding *et al.* using tilted-angle standing surface acoustic waves, and they achieved 84% purity with a throughput of 2 $\mu\text{l}/\text{min}$ (Table I).⁵⁷ In later work, the throughput has been increased by an order of magnitude to 20 $\mu\text{l}/\text{min}$ through device optimization.⁴³ Acoustofluidic-based separation has excellent biocompatibility in terms of maintaining the physiological state of the sorted cells.^{66,67} It utilizes similar acoustic power intensities and frequencies as those used in ultrasonic imaging, which has proven to be extremely safe. Also, it does not require modification of the surrounding media or labeling for separation. Nawaz *et al.* have demonstrated a positive separation technique using acoustofluidic FACS that separated fluorescently-labeled HeLa cells from unlabeled cells with a purity of 92.3% and a throughput of 20 $\mu\text{l}/\text{min}$ [Fig. 2(c)] (Table I). Moreover, 99.18% of the sorted cells remained viable after sorting, demonstrating the high biocompatibility of this acoustofluidic method (Table I).⁵⁶

III. CELL LYSIS

After the sorting of target cells, the genetic materials within the cell membrane must be accessed. First, the cell must be lysed to release its intracellular contents. Then, the genetic materials should be enriched and separated to prevent interference from other complex background molecules. In a conventional protocol, cell lysis and DNA extraction require multiple steps such as the mixing of buffers, followed by a series of centrifugation, heating, dehydration, and then resuspension.⁶⁸ Such a lengthy protocol introduces many issues, such as low throughput, the risk of cross-contamination, the requirement for a large amount of costly reagents, and the need for highly trained technicians. Several commercial kits from companies such as Qiagen, GE Healthcare, and

Thermo Fisher Scientific have been developed to minimize the time and cost. However, they still require bulky instruments such as centrifuges and heating blocks. Implementation of cell lysis and DNA extraction in a microfluidic device has the following anticipated benefits: it requires a small volume of reagent and hence is inexpensive; there is less risk of contamination due to the simple, disposable designs; and it is convenient to integrate with upstream sorting and downstream analysis modules.⁶⁹ There are four major microfluidic-based methods to lyse a cell: (1) mechanical lysis,⁷⁰ (2) thermal lysis,⁷¹ (3) electrical lysis,⁶⁹ and (4) chemical lysis.⁷² However, the chemical lysis methods will not be reviewed here since the chemical detergents used for lysis may interfere with downstream analysis, and hence these methods often require the implementation of additional washing steps which limit their compatibility with an integrated microfluidic system.⁶⁸ We have outlined recent advancements in each of the remaining types of lysis methods and their applicability in integrated microfluidic devices.

Mechanical lysis utilizes shear stress and frictional force, which arises during physical contact between embedded microstructures and the cells, to disrupt cell membranes. Di Carlo *et al.* have reported reagent-less mechanical lysis of cells in a continuous flow.⁷⁰ Here, nano-knives with sharp-edges have been fabricated inside the channel using a deep reactive ion etch (DRIE) on silicon. The distance between the neighboring nano-knife structures is about $3\text{ }\mu\text{m}$, which is too small for a cell to squeeze through easily and hence the shear force tears apart the membrane structures. The device was operated at a throughput of $300\text{ }\mu\text{l/min}$ for optimal lysis efficiency (Table I). Another example is based on bead beating. Kim *et al.* have demonstrated lysis of various cells using a compact disc with microchannels packed with silica beads.⁷³ As the disc was spun in an alternating manner in the clockwise and counterclockwise directions repeatedly, the sudden acceleration changes caused the silica beads to mechanically grind the cells apart. This method took 6 s for the entire operation to process a $300\text{ }\mu\text{l}$ volume sample and showed an efficiency (65%) comparable to a conventional lysis protocol (Table I). Hwang *et al.* have improved bead-beating-based cell lysis further by utilizing a vibrating polydimethylsiloxane (PDMS) membrane which acts as an actuator for inter-bead collisions [Fig. 3(a)].⁷⁴ They have demonstrated that within 3 min the device could extract a comparable amount to conventional benchtop methods of DNA from a 1 ml sample (Table I). This method is more compatible with downstream elements due to the simple actuator design and driving mechanism. And, it does not chemically alter the state of the analytes.

In electrical cell lysis techniques, a strong electric field is often applied to destabilize the membrane structure, although the exact mechanism is not yet fully understood.⁷⁵ Electrical lysis particularly benefits from miniaturization because the reduced distance between the electrodes allows for the generation of high electric field gradients with a lower voltage input.⁷⁶ For successful lysis of a cell, the electric potential gradient across the membrane should be high enough ($2\text{--}10\text{ kV cm}^{-1}$) to create irreversible pores^{77–79} but low enough ($\sim 1.3\text{ V}$) to prevent electrolysis of the buffer solution which will result in bubble formation near electrodes.⁸⁰ Although a direct current (DC) electric field assures more effective cell lysis as it maintains the field strength above the threshold potential, an alternating current (AC) electric field is favored to minimize bubble formation.⁸¹ The AC frequency should be carefully chosen to maintain the membrane potential above the threshold potential long enough to assure effective cell lysis. A continuous-flow cell lysis device utilizing an AC electric field has been developed by Mernier *et al.*⁷⁹ A liquid electrode was fabricated in a direction perpendicular to the longitudinal channel axis and generated a homogeneous electrical field (2.7 kV cm^{-1}) over the entire channel height. This device demonstrated an extremely high lysis efficiency (99%) using 55 V peak-to-peak (V_{pp}) at a frequency of 50 kHz (Table I). The majority of electrical lysis devices utilize planar electrodes. However, the electric field decays exponentially with distance from the plane of the electrode and thereby limits the effective volume where cell lysis occurs. Lu *et al.* employed cylindrical electrodes,⁸² which are capable of generating an electric field strength of $2,000\text{ kV cm}^{-1}$. They found that the volume in which the electric field is sufficient to lyse the cell was about 4 times larger than that of the planar electrodes (Table I). A more direct method has been developed to improve charge transfer from the electric field.⁷⁸ Cell lysis was performed using the corona discharge between micro-electrodes inside a microfluidic channel and initiated by a handheld corona device [Fig. 3(b)]. The corona discharge generated a voltage in the order of kV but with a minimal current, which allowed for efficient cell lysis without bubble

FIG. 3. Microfluidic-based cell lysis. (a) Microfluidic-based bead beating device for mechanical cell lysis.⁷⁴ The pneumatic vibration of polydimethylsiloxane (PDMS) actuates glass beads to induce vigorous collision and shear stress for mechanical cell lysis. Reprinted with permission from K.-Y. Hwang *et al.*, *Lab. Chip* **11**(21), 3649–3655 (2011). Copyright 2011 Royal Society of Chemistry Publishing.⁷⁴ (b) On-chip electrolysis using a hand-held corona device.⁷⁸ The microelectrode is pre-patterned in the microfluidic channel. Strong electric discharge from the handheld corona device generates a strong electric field around the microelectrode for cell lysis. Reprinted with permission from C. Escobedo *et al.*, *Lab. Chip* **15**(14), 2990–2997 (2015). Copyright 2015 Royal Society of Chemistry Publishing.⁷⁸ (c) The schematic of a microreactor integrated with a heater and a temperature sensor for single-step thermal lysis of a cell and subsequent PCR.⁸⁵ Reprinted with permission from C. Ke *et al.*, *Sens. Actuators B* **120**(2), 538–544 (2007). Copyright 2007 Elsevier.⁸⁵ (d) Microfluidic thermal lysis using a wireless induction coil.⁸⁶ An alternating magnetic field from the magnetic coil generates a thermal response at the heating unit inside the chip for thermal lysis. Reprinted with permission from S.-K. Baek *et al.*, *Lab. Chip* **10**(7), 909–917 (2010). Copyright 2010 Royal Society of Chemistry Publishing.⁸⁶

formation. Electrical lysis has a multitude of advantages in μ TAS applications as it provides a high lysis efficiency without using chemical reagents and has minimal effects on molecules lysed from the cells. Also, with sufficiently high electric field strength, the time required for lysis can be greatly reduced to the $\sim$ ms range. This is particularly advantageous for single cell analysis. Moreover, its simple operation makes it an attractive option for device integration. However, most electrical lysis techniques operate in a non-continuous mode which makes it challenging to integrate them with upstream and downstream microfluidic components.⁶⁹

Thermal lysis is another standard method for cell lysis. It involves using elevated temperatures to disrupt cell membranes and to denature protein structures.⁸³ Due to its highly destructive nature, it is often used in methods probing DNAs/RNAs which are less likely to be damaged by the heat treatment. For example, Waters *et al.* have developed an integrated microfluidic chip for the detection of target DNA fragments (Table I).⁸⁴ This chip was capable of sequential cell lysis, DNA amplification, and electrophoretic DNA fragment detection. However, their entire microchip was placed in a conventional thermocycler to lyse the cells and therefore did not particularly benefit from the rapid heating profiles possible due to miniaturization. Later, Ke *et al.* reported an array for single-step cell lysis in a PCR device with good thermal isolation [Fig. 3(c)].⁸⁵ They microfabricated platinum heating elements and temperature sensors below each micro-reactor chamber. This allowed for independent heating and cooling of each micro-reactor, with heating and cooling rates of 11 and 2.7 °C/s, respectively. However, this device was only able to process 3.5 μ l of sample in 32 min, and hence the throughput was limited compared to mechanical lysis methods. Also, wireless induction-based heating has been employed for thermal cell lysis [Fig. 3(d)].⁸⁶ In this

approach, a hexagonal nickel heating element was fabricated in a microfluidic channel using soft-lithography techniques. Then, an external alternating magnetic field was applied via an induction coil for heating. This device was capable of raising the temperature up to 160 °C within 5 s and showed lysis efficiency comparable to ultrasonic methods. This method was capable of processing 70 μ l sample in 8 s (Table I).

IV. EXTRACTION AND AMPLIFICATION

After the cells are lysed, DNAs must be purified from the other cellular components that might act as interferences in a later downstream analysis. Purification of DNAs has been demonstrated using various microstructures such as silica microbeads,⁸⁷ functionalized magnetic nanoparticles,⁸⁸ and micro-pillars.⁸⁹ One of the most popular methods is solid phase extraction (SPE). Most commercial DNA extraction kits are based on this method. SPE uses microfluidic channels packed with silica beads. As the cell lysate flows into the device and mixes with high ionic strength buffers, DNA molecules are adsorbed onto the surface of the silica beads due to electrostatic attraction while other background molecules are washed away. Then, the adsorbed DNA molecules are eluted upon the application of low ionic strength buffers.⁶⁸ Tian *et al.* have demonstrated extraction of DNA from white blood cells using a silica-bead-packed capillary array with a purification efficiency (70%) comparable to the conventional method (e.g., QiagenAMP) but with a much shorter processing time (10 min).⁹⁰ The flow rates for loading, washing, and eluting samples ranged from 2.6 ml/min to 14.2 ml/min (Table I). The advantage of this method is that it only required a single mixing step before the purification step, and the quality of the eluted DNA sample was sufficient for subsequent PCR analysis. One limitation of this approach was the loss of silica particles into the flow during the purification process. To further refine this technology, Breadmore *et al.* immobilized the silica particles in a sol-gel matrix inside the microfluidic channel, which acts as a glue between the particles, and it resulted in better intra- and inter-chip reproducibility.⁹¹ The device was capable of extracting DNAs at a flow rate of 4.16 μ l/min (Table I).

Reedy *et al.* developed an SPE chip utilizing 3D poly(methyl methacrylate) (PMMA) posts functionalized with chitosan [Fig. 4(a)].⁹² These PMMA posts were designed to have many trenches and hence exhibited a comparable surface area to the silica beads. However, their surfaces were coated with chitosan which is known to exhibit pH-induced DNA binding. The DNAs from whole blood samples were captured by flushing the device with an acidic washing buffer (pH 5) at a flow rate of 1.6 μ l/min to facilitate the DNA-chitosan interaction. The captured DNAs were subsequently eluted at pH 9. While the extraction efficiency was ~47.8%, which was comparable to conventional methods, this method is anticipated to have higher reproducibility due to the greater control over the binding surface area between the chips (Table I).⁹²

Magnetic nanoparticles have also been employed in SPE techniques due to the ease of manipulating the external magnetic field. While the solid phase in the aforementioned SPE methods is static inside the channel, Duarte *et al.* have developed a dynamic SPE method utilizing magnetic nanoparticles.⁸⁸ The channels were filled with silica nanoparticles with magnetic iron cores. The device was operated at a flow rate of 7 μ l/min. The applied external magnetic field caused agitation of these magnetic nanoparticles to maximize their interaction with the liquid sample and increased the extraction yield with a 15 times higher elution concentration compared to the macroscale method (Table I). Also, Karle *et al.* have demonstrated DNA extraction of *E. coli* using phase-transfer magnetophoresis in a continuous fashion [Fig. 4(b)].⁹³ Their device consisted of three channels for the binding, washing, and eluting buffer flows, respectively, which were joined at the phase transfer junction. The superparamagnetic particles were mixed with the cell lysate and flowed through the binding buffer channel. The rotating magnet at the center drew the superparamagnetic nanoparticles with the bound target DNAs toward the elution buffer channel where the DNAs were then separated from the superparamagnetic nanoparticles. This method achieved a throughput of 45 μ l/min and a 147% higher DNA recovery rate when compared to batch methods (Table I).

Prior to detection, the extracted DNAs are often subjected to an amplification step because the amount of DNAs present in a typical microfluidic sample volume is extremely small, particularly for single-cell analysis applications. The typical PCR requires cycling between three different

FIG. 4. Microfluidic extraction platforms. (a) Schematic design (top) and scanning electron microscopy (SEM) image (bottom) of a microfluidic DNA extraction device utilizing chitosan-coated poly(methylmethacrylate) (PMMA) posts. DNA molecules in the cell lysate bind and release with the chitosan coating in a pH-dependent manner.⁹² Reprinted with permission from C. R. Reedy *et al.*, Lab. Chip 11(9), 1603–1611 (2011). Copyright 2011 Royal Society of Chemistry Publishing.⁹² (b) Schematic view of continuous phase-transfer magnetophoresis. DNA molecules in the sample are first bound to the magnet and injected into the device. The central permanent magnet is rotated in the opposite direction to the flow to transfer magnetic beads from the binding and lysis buffer, washing buffer, and elution buffer in sequence. Target DNAs are released from magnetic beads and collected at the eluate outlet.⁹³ Reprinted with permission from M. Karle *et al.*, Lab. Chip 10(23), 3284–3290 (2010). Copyright 2010 Royal Society of Chemistry Publishing.⁹³

temperatures to perform the following steps: denaturation of double-stranded DNAs at 95 °C, primer annealing at 45 °C, and extension of the DNAs at 72 °C. In principle, the extent of the amplification can be controlled merely by repeating the number of temperature cycles.

The first attempt at miniaturizing PCR dates back to the 1990s when a micro-chamber structure was employed for a benchtop PCR device.⁹⁴ However, this approach has a number of limitations.⁹ For example, the entire structure was subjected to thermocycling which resulted in an uneven thermal distribution due to conduction differences at interfaces and in different materials. In addition, thermocycling time was prolonged (~3 min) (Table I). Also, the increase in the surface-to-volume ratio between the PCR mixture and chamber resulted in significant adsorption of DNA molecules onto the wetted chamber surfaces, which eventually led to PCR inhibition. Therefore, its application was limited to small fragments of DNAs.⁹⁵

Later, the concept of continuous-flow PCR was developed by Kopp *et al.*⁹⁶ The PCR mixture was transported back and forth across regions with different fixed temperatures (95 °C for melting, 66 °C for annealing, and 70 °C for extension) for 20 cycles to achieve thermocycling [Fig. 5(a)]. The time for each cycle was modulated from 4.5 s/cycle to 56.4 s/cycle by changing the flow rate (Table I). Since the volume of the thermal mass of the PCR mixture was restricted by the dimensions within a specific section of the microchannel, the thermal inertia was considerably smaller than in the case of a micro-chamber PCR, which led to a significant reduction in the time required to ramp a sample to the appropriate temperature.^{9,96} However, unlike the micro-chamber PCR, the total number of thermal cycles in continuous-flow PCR was pre-determined by the channel design since the device operates in a flow-through mode. This drawback has been resolved by a closed-loop, continuous-flow PCR developed by Liu *et al.*⁹⁷ In this design, a single complete PCR cycle could be achieved in 20–30 s with the flexibility to modulate the total number of thermal cycles on a single chip design (Table I). Recently, Moschou *et al.* have demonstrated an even shorter thermocycling time of 10 s through the integration of a serpentine copper resistor layout (Table I).⁹⁸ The copper resistor also acts as a temperature sensor for feedback control on a polyimide microfluidic device. The polyimide ensures excellent thermal isolation between each temperature zone and temperature uniformity within a zone. However, the problems of surface adsorption and contamination remain unresolved due to the increased surface area to volume ratio. These problems could contribute to inhibition of the PCR. Moreover, the fluidic flow will naturally establish a parabolic velocity gradient in a continuous-flow PCR. Therefore, the fraction of the PCR mixture

FIG. 5. Microfluidic amplification platforms. (a) Schematic design of continuous PCR. A sample and PCR reagent are injected into a microfluidic chip. In the fluid channel, the injected solution is moved between the denaturation zone (95 °C), annealing zone (77 °C), and extension zone (60 °C). The whole chip incorporates 20 cycles of amplification.⁹⁶ Reprinted with permission from M. U. Kopp *et al.*, *Science* **280**(5366), 1046–1048 (1998). Copyright 1998 Science Publishing Group.⁹⁶ (b) Optical image (top) and schematic design (bottom) of a radial droplet PCR device. The device contains an oil inlet (A) that joins two aqueous inlet channels (B1 and B2) to form droplets at a T-junction (C). The droplets pass through the inner circles (500 μ m wide channels) in the hot zone (D) to ensure initial denaturation of the template and travel on to the periphery in 200 μ m wide channels where primer annealing and template extension occur (E). The droplets cycle between the center and the periphery for 34 cycles until they are collected at the outlet.¹⁰⁰ Reprinted with permission from Y. Schaeferli *et al.*, *Anal. Chem.* **81**(1), 302–306 (2008). Copyright 2008 American Chemical Society Publications.¹⁰⁰

closest to the channel surface will have a longer residence time compared to the fraction at the flow center line.

The PCR inhibition problem has been resolved through the recent development of droplet-based PCR.⁹⁹ Typically, a fluid droplet containing samples and PCR reagents is completely surrounded by an immiscible oil carrier layer which prevents carryover contamination between the droplets and sample adsorption to the channel walls. Therefore, each droplet acts as an individually isolated reactor. Moreover, due to the inherently small volume ($\sim$ picoliter), there are very few copies of nucleotides in each droplet, which significantly reduces the interference from homologous sequences. Additionally, the thermal mass of each individual droplet is significantly reduced compared to the previous methods, which guarantees more uniform heating across the droplet. A high-throughput droplet PCR device has been developed by Schaeferli *et al.*¹⁰⁰ In this design, the fluidic channel was fabricated on a circular PCR chip and the center of the chip was heated to generate a temperature gradient across the center (denaturation zone) and the periphery (annealing and extension zones) [Fig. 5(b)]. The droplets were generated at the T-junction and subsequently passed through a fluidic channel, relaying the droplets between the center and the periphery of the chip up to 34 cycles in 17 min (Table I). This method demonstrated that even a single copy of DNA per droplet could be successfully amplified. More detailed reviews of droplet PCR-based approaches can be found elsewhere.^{9,99}

V. DETECTION AND ANALYSIS

The final step of the genetic analysis requires the identification of the sequence and abundance of the amplified DNA. A number of conventional biochemical DNA detection methods have been translated to microfluidic platforms such as capillary electrophoresis (CE),¹⁰¹ FISH,¹¹ electrochemical detection,¹⁰² and microarrays.¹⁰³

Capillary electrophoresis (CE) was one of the first methods adopted in microfluidics in order to leverage the advantages that microfluidic platforms offer. Amplified DNA molecules are usually labeled with fluorescent dyes which are intercalated into the bases of double-stranded DNA (dsDNA). Upon the application of an electric field, the DNA fragments migrate along the channel at different velocities depending on their sizes. While a number of detection technologies have been coupled with CE for the detection of DNA, laser-induced fluorescence (LIF) is the most commonly used detection method due to its superior selectivity and sensitivity. Kotani *et al.* have integrated microchip CE with a dual-color LIF system to detect sporadic mutations of p53 which are often masked by an excess of wild-type DNA.¹⁰⁴ They used endonuclease V which cleaves mismatch sites in dsDNA and AK16D DNA ligase which repairs any undesired cleavage in matched sites to generate fragments of mutated DNA. Finally, the fluorescence intensity of each band on the electropherogram was measured to determine the position of the mutations. Also, Zhang *et al.* have reported a similar CE-LIF system to detect low-abundance KRAS mutations which are frequently found in colorectal cancers [Fig. 6(a)].¹⁰⁵ While this method is straightforward and easy to integrate, only a limited amount of information (i.e., fragment size) from the analyzed DNA is available and cannot be used to report information about the sequence of base pairs of interest. Also, this method is considered to be a qualitative method because the precise quantity of the target DNA is often difficult to determine. Moreover, the miniaturization and integration of fluorescence-based systems are not readily achievable as they require a laser source and optical focusing and detection components.¹⁰⁶

Electrochemical detection is an attractive alternative due to its compatibility with microfabrication techniques and the lack of a need for complicated detection and feedback control systems. Usually, a customized probe of oligonucleotide sequences, which are complementary to the target DNA, are immobilized onto thin-film metal electrodes patterned on a substrate. Hybridization of the target sequence with the probe generates electrons that can be measured by a change in voltage, current, or impedance. Lai *et al.* have reported a reusable and reagent-less electrochemical sensor for PCR amplicons.¹⁰⁷ The nucleotide sequences used as probes are labeled with a redox indicator, methylene blue, and are immobilized on the gold electrode surface. The change in conformation of the probe in the presence of the target DNA sequence changes the relative distance between the methylene blue and the electrode surface, thereby changing the reduction potential. This reduction potential change can be measured. Also, they have demonstrated that a simple washing step with distilled water was sufficient to reuse this electrochemical sensor after each detection.

One of the limitations with electrochemical sensors is the need to identify the nucleotide sequence for the probes before the fabrication stage. Labib *et al.* have reported a novel electrochemical sensor platform which can be universally applied to detect any micro-RNA (miRNA) sequence without needing to change the immobilized probe [Fig. 5(b)].¹⁰⁸ The sensor platform consists of a universal interface probe (UIP) immobilized on the sensor surface and two adaptor DNA sequences in solution. Each adaptor sequence has a nucleotide sequence complementary to the UIP and, at a different location, a sequence that is complementary to the target. Therefore, the UIP and two adaptors form a four-way junction in the presence of the target miRNA. This suggests a potential for a universal DNA sensing platform by solely changing the sequence of adaptors. Recently, Islam *et al.* have demonstrated an ultra-sensitive electrochemical sensor platform which eliminates the need for amplification prior to detection.¹⁰⁹ Their protocol consists of three parts. First, the target RNAs from cancerous cells are extracted using magnetic nanoparticles. This is followed by heating to release the isolated RNAs. The free RNA molecules are then directly adsorbed onto the sensor surface and are detected through the reduction of $[\text{Fe}(\text{CN})_6]^{4-/3-}$. This method has shown a limit-of-detection of 1.0 pM with an inter-assay reproducibility of >95%. This method demonstrated 25 times higher sensitivity than previously developed methods that involve ligation-dependent probe amplification prior to the electrochemical detection.¹¹⁰

While the aforementioned technologies are limited to a single or a few sets of target sequences, microarray-based techniques allow for the parallel detection of a vast number of different oligonucleotide sequences. With microarrays, a set of predefined probe oligonucleotides are printed onto a substrate in a spatially localized fashion.¹¹¹ The major advantage of this approach lies with its multiplexing capability to quickly identify nucleotide sequences of interest. For

FIG. 6. Microfluidic-based detection platforms. (a) A capillary electrophoresis device coupled with laser-induced fluorescence (LIF) for DNA detection.¹⁰⁵ Target DNAs are amplified by mismatched primers followed by *Mva* I digestion to generate different fragment patterns from wildtype, mutant, and heterozygous amplicons. The amplicons are injected into micro-capillary electrophoresis chips to distinguish different fragment patterns. Reprinted with permission from H. Zhang *et al.*, PLoS ONE 8(1), e54510 (2013). Copyright 2013 Public Library of Science.¹⁰⁵ (b) A four-way-junction electrochemical sensor platform.¹⁰⁸ The target microRNA (miRNA) form a four-way junction with a universal interface probe (UIP) on the sensor surface and two DNA adaptor strands labeled with biotin. Subsequently, streptavidin binds to the four-way junction to amplify the interfacial resistance, which allows for sensitive detection of the target miRNA. Reprinted with permission from M. Labib *et al.*, Anal. Chem. 85(20), 9422–9427 (2013). Copyright 2013 American Chemical Society Publications.¹⁰⁸ (c) Self-assembled, picoliter droplet microarray.¹¹⁴ The target DNA solution is placed on the sensor surface for hybridization and detection. The DNA probe-target duplexes are labeled with quantum dots for observation. Reprinted with permission from T. M. Yen *et al.*, ACS Nano 9(11), 10655–10663 (2015). Copyright 2015 American Chemical Society Publications.¹¹⁴

example, Ishkanian *et al.* have demonstrated a high-density DNA microarray capable of covering the entire human genome with 32 433 probes.¹¹² However, conventional microarrays are diffusion-limited because the diffusion of DNA could take up to 24 h to travel 1 mm under static conditions. The slow diffusion speed affects the overall sensitivity and the limit-of-detection of a microarray-

based approach. There have been a number of attempts to address this diffusion limitation. For example, Martins *et al.* have utilized an electrokinetic concentrator to enhance DNA hybridization.¹¹³ They fabricated a microfluidic channel with poly(3,4-ethylenedioxythiophene)-poly-(styrenesulfonate) (PEDOT:PSS) which serves as a cation-selective membrane. Upon the application of a voltage, the membrane works as a sieve collecting anionic DNA molecules and increases the DNA concentration by up to 800-fold. This method showed a 10-fold increase in hybridization time with a 2-fold increase in the sensitivity compared to conventional microarrays. Also, Yen *et al.* have demonstrated a picoliter-droplet-based microarray with ultra-high sensitivity and a hybridization time as short as 5 min [Fig. 6(c)].¹¹⁴ Here, they have patterned DNA probes on a circular hydrophilic region surrounded by an ultra-hydrophobic region. Upon the introduction of the sample solution, a picoliter droplet self-assembles on top of the hydrophilic region. The concentration of DNA in each droplet increases by 200 000 fold as the droplet starts to evaporate, resulting in a maximum sensitivity of 50 aM.

VI. INTEGRATED SYSTEMS

With the advances made possible by these aforementioned techniques, a number of researchers have made significant progress toward the realization of integrated lab-on-a-chip (LOC) devices for the molecular analysis of cells.¹¹⁵ Zheng *et al.* have demonstrated size-based selective isolation of CTCs with subsequent on-chip electrical cell lysis for the downstream genetic analysis.¹¹⁶ They used microfabrication techniques to pattern electrodes mid-way through each pore. The differences in size and deformability between CTCs and red blood cells allowed for selective separation while the close contact between the cell and electrode, as it passed through a pore, allowed for lysis at voltages as low as 5 V peak-to-peak (V_{pp}). This method showed a CTC recovery rate of 90% within 10 min and a successful elution of the cell lysate from the membrane for downstream, off-chip, PCR amplification. While this method is straightforward and easy to fabricate, integration into the microfluidic channel for complete on-chip analysis would be difficult due to the inherent structure of the membrane. Later, Geng *et al.* demonstrated DNA extraction by electroporation on a microfluidic channel using multiple pneumatic valves [Fig. 7(a)].¹¹⁷ Chinese hamster ovary cells were injected into a microfluidic channel packed with magnetic beads. The cells were physically trapped in the lysis chamber by a valve and subsequently lysed upon the application of electrical pulses at a frequency of 0.1 Hz. Then, the cell lysate and bead mixture were transferred to a washing chamber, where oscillating pressure was applied to facilitate adsorption of DNAs onto the magnetic beads. Finally, the extracted DNA-bead complexes were magnetically separated and eluted for off-chip PCR analysis.

Recently, more sophisticated microfluidic platforms capable of on-chip analysis have been reported. For example, Jha *et al.* have demonstrated an integrated microfluidic chip consisting of three discrete modules: an electrochemical lysis chamber, an amplification reactor, and a CE-based detection element.¹¹⁸ First, the injected cells were lysed by hydroxide ions generated by the application of DC voltage. The use of hydroxide ions permanently denatured the cell membrane without a need for the excessive voltages often associated with DC-based lysis methods. Subsequently, the DNAs were amplified by 25-fold in a continuous-flow PCR using integrated thin-film microheaters. Finally, the target DNAs were separated and detected from the amplicon population using a CE-amperometric detection element on the microchip. Despite the unspecific PCR amplification, this method showed great potential for point-of-care applications as the entire operation, from lysis to detection, used a DC. Thus, it could easily be battery powered. Similar work has been reported by Le Roux *et al.* which consists of a thermal lysis element, a microliter, non-contact PCR, and a CE-LIF detection element.¹¹⁹ While CE-based chips possess advantages in portability and high sensitivity, the difficulty in fabricating an array capable of multiplexed processing has been a major limitation.

A number of researchers have reported fully integrated chip-based microarray solutions. For example, Summerer *et al.* have demonstrated a simple PCR-microarray chip for the multiplexed detection of human genomic DNA with an excellent sensitivity requiring less than 100 copies.¹²⁰ The sample lysate and PCR reagent were directly mixed off-chip and then loaded onto the

FIG. 7. Fully integrated microfluidic systems. (a) An integrated microfluidic platform for on-chip electrical lysis and DNA extraction.¹¹⁷ Cells are electrically lysed upon application of direct current. The lysate is transferred to a subsequent module where an alternating pressure is applied to mix the lysate and DNA-capturing magnetic beads for DNA extraction. Reprinted with permission from T. Geng *et al.*, *Anal. Chem.* **84**(21), 9632–9639 (2012). Copyright 2012 American Chemical Society Publications.¹¹⁷ (b) A fully integrated chip for electrochemical lysis and mRNA profiling.¹²³ Circulating tumor cells (CTCs) labeled with magnetic nanoparticles are captured by the trapping structure and subsequently lysed through electrochemical lysis. Release mRNAs are flushed to the sensing module for electrochemical detection. Reprinted with permission from R. M. Mohamadi *et al.*, *Anal. Chem.* **87**(12), 6258–6264 (2015). Copyright 2015 American Chemical Society Publications.¹²³ (c) Fully integrated DC driven device for cell lysis, nucleic acid enrichment, and detection. The integrated platform consists of three units including a pretreatment unit for cell lysis, a pre-concentration unit for the concentration of isolated nucleic acids, and sensing unit for specific detection of target nucleic acid.¹²² Reprinted with permission from Z. Slouka *et al.*, *Talanta* **145**, 35–42 (2015). Copyright 2015 Elsevier.¹²²

microarray platform. The sample had already been amplified by a conventional thermocycler in the presence of biotin-labeled free nucleotides which were incorporated into the amplified product. Subsequently, the amplified products were hybridized to a pre-patterned microarray probe. After a washing step, the target DNAs were attached to a fluorescently-labeled streptavidin. The entire procedure was carried out in a single module. Although this method was not fully automated, such a simple design could be easily integrated with an upstream sample preparation module for fully automated analysis. Later, Roy *et al.* reported a fully automated nucleic acid assay using a lab-on-disc system.¹²¹ The microfluidic disc was able to perform cell lysis by bead beating, PCR amplification, amplicon digestion, and microarray analysis. Unlike other microfluidic technologies, this method relied on the centrifugal force generated by the spinning disc for the liquid transportation, thereby eliminating the need for additional fabrication layers, microvalves, and micropumps.

Most of the above-mentioned chips relied on PCR amplification due to the inherently scarce number of target DNAs. Several researchers have reported PCR-free integrated designs through the use of pre-concentration or ultra-sensitive detection platforms. For example, Slouka *et al.* have demonstrated a PCR-free integrated electrochemical detection device for oral cancer diagnosis [Fig. 7(c)].¹²² This device consists of three different ion-selective nanoporous membranes for pre-treatment, pre-concentration, and detection. The cells were chemically lysed in a pre-treatment chamber, and the negatively charged miRNA was selectively filtered through the first ion-selective membrane upon the application of a DC voltage. In the pre-concentration unit, a cation exchange membrane acted as an ionic plug, which generates electrophoretic motion in the negatively charged

miRNA while the fluid flow generates an opposite motion. The balance between these two motions resulted in the concentration of miRNA around the sensing membrane. The miRNA was hybridized at the sensing membrane which consisted of an anion exchange membrane labeled with a DNA probe. It was monitored through changes in the current-voltage (I-V) characteristics. Using this device, they successfully demonstrated detection of miRNA from a cancer cell sample within 30 min.

Mohamadi *et al.* reported an integrated microchip capable of performing a sample-to-answer process and consisting of only two elements for genetic analysis [Fig. 7(b)].¹²³ A whole blood sample containing spiked CTCs was injected along with anti-CTC superparamagnetic nanoparticles. The CTC capture and lysis chamber consisted of an array of X-shaped pillars which served two functions. First, they created a local region of retarded fluid flow which, in conjunction with an external magnetic field, efficiently trapped the bulky CTC-magnetic nanoparticle complexes. The device demonstrated a higher CTC capture efficiency (60%) and two orders-of-magnitude higher purity (97%) over the conventional gold-standard approaches. Moreover, it generated hydroxide ions for electrochemical lysis of the captured cells. The cell lysate was then transferred to a detection chamber which consisted of needle-like microelectrodes labeled with peptide nucleic acid probes for the detection of target mRNA. This device demonstrated that mRNA from as few as 2 CTCs in a whole blood sample could be detected.

VII. SUMMARY AND FUTURE PERSPECTIVES

The advances in microfluidic technologies have enabled complex biochemical assays to be performed in an automated, sensitive, precise, cost-effective, and high-throughput manner that were only previously possible with expensive and complicated bench-top instruments. The microfluidic-based modular approach to genomic analysis from sample preparation to the extraction, amplification, and detection of nucleic acids has matured significantly over the past two decades. There are still a number of roadblocks which have to be resolved before these devices can become practical for routine clinical use. There have only been a handful of innovative examples of fully-integrated microfluidic devices that have translated various biochemical protocols out of the clinical laboratories and toward the point-of-care. Therefore, it is anticipated that future research will be more focused on the integration of these modular components into single devices. Also, the majority of these devices have only been demonstrated with simplified samples containing known cells or DNA fragments spiked into controlled buffers. It remains unclear whether their performance would be downgraded if more clinically relevant samples were tested.

While these devices target genomic biomarkers within the cell, their application could be extended to target cell-like biomarkers such as exosomes. Exosomes are 30~150 nm sized vesicles secreted by virtually all types of cells. They are known to contain proteins and DNA/RNA markers which can be correlated back to the condition of their originating cells. A growing body of research suggests that vesicles such as exosomes are the next-generation of diagnostic biomarkers due to their abundance while requiring less invasive sample collection processes.¹²⁴ While their size is significantly smaller than that of cells, they share a similar workflow for analysis. Researchers have already reported essentially similar microfluidic platforms used for cell analysis being applied to exosome analysis.^{125–127} Therefore, it is anticipated that a majority of these technologies can be translated into exosome-based diagnostics.

As these microfluidic modules and integrated devices become more ubiquitous, it will bring about a paradigm shift in expanding the number of convenient and compact tools available to perform routine diagnosis and for efficient, long-term monitoring of the progression of chronic diseases.

ACKNOWLEDGMENTS

We acknowledge support from the National Institutes of Health (R01 HD086325, R44 HL126441, R01 AI120560, and R44GM125439) and the National Science Foundation (IIP-1534645).

- ¹X. Mao and T. J. Huang, *Lab. Chip* **12**(8), 1412–1416 (2012).
- ²H. Andersson and A. Van den Berg, *Sens. Actuators B* **92**(3), 315–325 (2003).
- ³R. N. Zare and S. Kim, *Ann. Rev. Biomed. Eng.* **12**, 187–201 (2010).
- ⁴C. W. Shields IV, C. D. Reyes, and G. P. López, *Lab. Chip* **15**(5), 1230–1249 (2015).
- ⁵F. Cui, M. Rhee, A. Singh, and A. Tripathi, *Ann. Rev. Biomed. Eng.* **17**, 267–286 (2015).
- ⁶T. H. Schulte, R. L. Bardell, and B. H. Weigl, *Clin. Chim. Acta* **321**(1), 1–10 (2002).
- ⁷V. Srinivasan, V. K. Pamula, and R. B. Fair, *Lab. Chip* **4**(4), 310–315 (2004).
- ⁸A. H. Ng, U. Uddayasankar, and A. R. Wheeler, *Anal. Bioanal. Chem.* **397**(3), 991–1007 (2010).
- ⁹Y. Zhang and P. Ozdemir, *Anal. Chim. Acta* **638**(2), 115–125 (2009).
- ¹⁰B. M. Paegel, R. G. Blazej, and R. A. Mathies, *Curr. Opin. Biotechnol.* **14**(1), 42–50 (2003).
- ¹¹K.-J. Kao, C.-H. Tai, W.-H. Chang, T.-S. Yeh, T.-C. Chen, and G.-B. Lee, *Biosens. Bioelectron.* **69**, 272–279 (2015).
- ¹²G. Hybarger, J. Bynum, R. F. Williams, J. J. Valdes, and J. P. Chambers, *Anal. Bioanal. Chem.* **384**(1), 191–198 (2006).
- ¹³S.-I. Hori, A. Herrera, J. J. Rossi, and J. Zhou, *Cancers* **10**(1), 9 (2018).
- ¹⁴L. Ying and Q. Wang, *BMC Biotechnol.* **13**(1), 76 (2013).
- ¹⁵F. D. Ottery, *Cancer Pract.* **2**(2), 123–131 (1994).
- ¹⁶P. A. Newcomb, R. G. Norfleet, B. E. Storer, T. S. Surawicz, and P. M. Marcus, *J. Natl. Cancer Inst.* **84**(20), 1572–1575 (1992).
- ¹⁷U. T. O. E. D. Of and B. C. Group, *Lancet* **332**(8608), 411–416 (1988).
- ¹⁸J. Cuzick, A. DeCensi, B. Arun, P. H. Brown, M. Castiglione, B. Dunn, J. F. Forbes, A. Glaus, A. Howell, and G. von Minckwitz, *Lancet Oncol.* **12**(5), 496–503 (2011).
- ¹⁹L. E. MacConaill, C. D. Campbell, S. M. Kehoe, A. J. Bass, C. Hatton, L. Niu, M. Davis, K. Yao, M. Hanna, and C. Mondal, *PLoS ONE* **4**(11), e7887 (2009).
- ²⁰E. Crowley, F. Di Nicolantonio, F. Loupakakis, and A. Bardelli, *Nat. Rev. Clin. Oncol.* **10**(8), 472 (2013).
- ²¹C. Alix-Panabières and K. Pantel, *Clin. Chem.* **59**(1), 110–118 (2013).
- ²²D. F. Carr, A. Alfirevic, and M. Pirmohamed, *Genes* **5**(2), 430–443 (2014).
- ²³K. Jain, *Pharmacogenomics* **1**(3), 289–307 (2000).
- ²⁴D. Braun, *Pharmacogenomics* **4**, 375–378 (2003).
- ²⁵A. J. Thompson, W. G. Newman, R. A. Elliott, S. A. Roberts, K. Tricker, and K. Payne, *Value Health* **17**(1), 22–33 (2014).
- ²⁶J. A. Johnson, K. E. Caudle, L. Gong, M. Whirl-Carrillo, C. M. Stein, S. A. Scott, M. Lee, B. F. Gage, S. E. Kimmel, and M. A. Perera, *Clin. Pharmacol. Ther.* **102**(3), 397–404 (2017).
- ²⁷A. A. S. Bhagat, H. Bow, H. W. Hou, S. J. Tan, J. Han, and C. T. Lim, *Med. Biol. Eng. Comput.* **48**(10), 999–1014 (2010).
- ²⁸P. T. Sharpe, *Methods of Cell Separation* (Elsevier, 1988).
- ²⁹T. R. Mosmann and S. Sad, *Immunol. Today* **17**(3), 138–146 (1996).
- ³⁰M. Munz, P. A. Baeuerle, and O. Gires, *Cancer Res.* **69**(14), 5627–5629 (2009).
- ³¹R. G. Austin, T. J. Huang, M. Wu, A. J. Armstrong, and T. Zhang, *Adv. Drug Deliv. Rev.* **125**, 132–142 (2018).
- ³²B. D. Plouffe, S. K. Murthy, and L. H. Lewis, *Rep. Prog. Phys.* **78**(1), 016601 (2014).
- ³³R. Gertler, R. Rosenberg, K. Fuehrer, M. Dahm, H. Nekarda, and J. R. Siewert, *Recent Results Cancer Res.* **162**, 149–156 (2003).
- ³⁴M. J. Tomlinson, S. Tomlinson, X. B. Yang, and J. Kirkham, *J. Tissue Eng.* **4**, 2041731412472690 (2013).
- ³⁵L. Wang, P. Balasubramanian, A. P. Chen, S. Kummar, Y. A. Evrard and R. J. Kinders, *Semin. Oncol.* **43**, 464–475 (2016).
- ³⁶M. C. Miller, G. V. Doyle, and L. W. Terstappen, *J. Oncol.* **2010**, Article ID 617421.
- ³⁷S. Moon, H. O. Keles, A. Ozcan, A. Khademhosseini, E. Hæggstrom, D. Kuritzkes, and U. Demirci, *Biosens. Bioelectron.* **24**(11), 3208–3214 (2009).
- ³⁸A. Y. Fu, C. Spence, A. Scherer, F. H. Arnold, and S. R. Quake, *Nat. Biotechnol.* **17**(11), 1109–1111 (1999).
- ³⁹L. A. Herzenberg, D. Parks, B. Sahaf, O. Perez, M. Roederer, and L. A. Herzenberg, *Clin. Chem.* **48**(10), 1819–1827 (2002).
- ⁴⁰S. Basu, H. M. Campbell, B. N. Dittel, and A. Ray, *J. Visual. Exp.* **41**, e1546 (2010).
- ⁴¹C. Liu and G. Hu, *Micromachines* **8**(3), 73 (2017).
- ⁴²D. R. Gossett, W. M. Weaver, A. J. Mach, S. C. Hur, H. T. K. Tse, W. Lee, H. Amini, and D. Di Carlo, *Anal. Bioanal. Chem.* **397**(8), 3249–3267 (2010).
- ⁴³P. Li, Z. Mao, Z. Peng, L. Zhou, Y. Chen, P.-H. Huang, C. I. Truica, J. J. Drabick, W. S. El-Deiry, and M. Dao, *Proc. Natl. Acad. Sci. U.S.A.* **112**(16), 4970–4975 (2015).
- ⁴⁴H. Amini, W. Lee, and D. Di Carlo, *Lab. Chip* **14**(15), 2739–2761 (2014).
- ⁴⁵J. Zhang, S. Yan, D. Yuan, G. Alici, N.-T. Nguyen, M. E. Warkiani, and W. Li, *Lab. Chip* **16**(1), 10–34 (2016).
- ⁴⁶D. Di Carlo, *Lab. Chip* **9**(21), 3038–3046 (2009).
- ⁴⁷H. W. Hou, M. E. Warkiani, B. L. Khoo, Z. R. Li, R. A. Soo, D. S.-W. Tan, W.-T. Lim, J. Han, A. A. S. Bhagat, and C. T. Lim, *Sci. Rep.* **3**, 1259 (2013).
- ⁴⁸M. G. Lee, J. H. Shin, C. Y. Bae, S. Choi, and J.-K. Park, *Anal. Chem.* **85**(13), 6213–6218 (2013).
- ⁴⁹J. Che, V. Yu, M. Dhar, C. Renier, M. Matsumoto, K. Heirich, E. B. Garon, J. Goldman, J. Rao, and G. W. Sledge, *Oncotarget* **7**(11), 12748 (2016).
- ⁵⁰J. Voldman, *Annu. Rev. Biomed. Eng.* **8**, 425–454 (2006).
- ⁵¹H. Morgan, M. P. Hughes, and N. G. Green, *Biophys. J.* **77**(1), 516–525 (1999).
- ⁵²J.-C. Baret, O. J. Miller, V. Taly, M. Ryckelynck, A. El-Harrak, L. Frenz, C. Rick, M. L. Samuels, J. B. Hutchison, and J. J. Agresti, *Lab. Chip* **9**(13), 1850–1858 (2009).
- ⁵³A. Alazam, I. Stiharu, R. Bhat, and A. N. Meguerditchian, *Electrophoresis* **32**(11), 1327–1336 (2011).
- ⁵⁴A. J. Chung and S. C. Hur, *Micro- and Nanomanipulation Tools* (John Wiley & Sons, Inc, 2015), Vol. 13.
- ⁵⁵M. Li and R. K. Anand, *J. Am. Chem. Soc.* **139**(26), 8950–8959 (2017).
- ⁵⁶A. A. Nawaz, Y. Chen, N. Nama, R. H. Nissly, L. Ren, A. Ozcelik, L. Wang, J. P. McCoy, S. J. Levine, and T. J. Huang, *Anal. Chem.* **87**(24), 12051–12058 (2015).

- ⁵⁷X. Ding, Z. Peng, S.-C. S. Lin, M. Geri, S. Li, P. Li, Y. Chen, M. Dao, S. Suresh, and T. J. Huang, *Proc. Natl. Acad. Sci. U.S.A.* **111**(36), 12992–12997 (2014).
- ⁵⁸L. Ren, Y. Chen, P. Li, Z. Mao, P.-H. Huang, J. Rufo, F. Guo, L. Wang, J. P. McCoy, and S. J. Levine, *Lab. Chip* **15**(19), 3870–3879 (2015).
- ⁵⁹J. Shi, H. Huang, Z. Stratton, Y. Huang, and T. J. Huang, *Lab. Chip* **9**(23), 3354–3359 (2009).
- ⁶⁰S. Li, L. Ren, P.-H. Huang, X. Yao, R. A. Cuento, J. P. McCoy, C. E. Cameron, S. J. Levine, and T. J. Huang, *Anal. Chem.* **88**(11), 5655–5661 (2016).
- ⁶¹Y. Chen, M. Wu, L. Ren, J. Liu, P. H. Whitley, L. Wang, and T. J. Huang, *Lab. Chip* **16**(18), 3466–3472 (2016).
- ⁶²S. Li, F. Ma, H. Bachman, C. E. Cameron, X. Zeng, and T. J. Huang, *J. Micromech. Microeng.* **27**(1), 015031 (2016).
- ⁶³L. Johansson, F. Nikolajeff, S. Johansson, and S. Thorslund, *Anal. Chem.* **81**(13), 5188–5196 (2009).
- ⁶⁴O. Jakobsson, C. Grenvall, M. Nordin, M. Evander, and T. Laurell, *Lab. Chip* **14**(11), 1943–1950 (2014).
- ⁶⁵J. Shi, S. Yazdi, S.-C. S. Lin, X. Ding, I.-K. Chiang, K. Sharp and T. J. Huang, *Lab. Chip* **11**(14), 2319–2324 (2011).
- ⁶⁶M. Wiklund, *Lab. Chip* **12**(11), 2018–2028 (2012).
- ⁶⁷X. Ding, S.-C. S. Lin, B. Kiraly, H. Yue, S. Li, I.-K. Chiang, J. Shi, S. J. Benkovic, and T. J. Huang, *Proc. Natl. Acad. Sci. U.S.A.* **109**(28), 11105–11109 (2012).
- ⁶⁸J. Kim, M. Johnson, P. Hill, and B. K. Gale, *Integr. Biol.* **1**(10), 574–586 (2009).
- ⁶⁹H. Lu, M. A. Schmidt, and K. F. Jensen, *Lab. Chip* **5**(1), 23–29 (2005).
- ⁷⁰D. Di Carlo, K.-H. Jeong, and L. P. Lee, *Lab. Chip* **3**(4), 287–291 (2003).
- ⁷¹C.-Y. Lee, G.-B. Lee, J.-L. Lin, F.-C. Huang, and C.-S. Liao, *J. Micromech. Microeng.* **15**(6), 1215 (2005).
- ⁷²B. P. Mun, S. M. Jung, S. Y. Yoon, S. H. Kim, J. H. Lee, and S. Yang, *Microfluid. Nanofluid.* **8**(5), 695–701 (2010).
- ⁷³J. Kim, S. H. Jang, G. Jia, J. V. Zoval, N. A. Da Silva, and M. J. Madou, *Lab. Chip* **4**(5), 516–522 (2004).
- ⁷⁴K.-Y. Hwang, S. H. Kwon, S.-O. Jung, H.-K. Lim, W.-J. Jung, C.-S. Park, J.-H. Kim, K.-Y. Suh, and N. Huh, *Lab. Chip* **11**(21), 3649–3655 (2011).
- ⁷⁵F. Apollonio, M. Liberti, P. Marracino and L. Mir, 2012 6th European Conference on Antennas and Propagation (EUCAP), 2012.
- ⁷⁶M. Khine, A. Lau, C. Ionescu-Zanetti, J. Seo, and L. P. Lee, *Lab. Chip* **5**(1), 38–43 (2005).
- ⁷⁷T. Y. Tsong, *Biophys. J.* **60**(2), 297–306 (1991).
- ⁷⁸C. Escobedo, S. Bürgel, S. Kemmerling, N. Sauter, T. Braun, and A. Hierlemann, *Lab. Chip* **15**(14), 2990–2997 (2015).
- ⁷⁹G. Memier, N. Piacentini, T. Braschler, N. Demierre, and P. Renaud, *Lab. Chip* **10**(16), 2077–2082 (2010).
- ⁸⁰S. Wang, X. Zhang, W. Wang, and L. J. Lee, *Anal. Chem.* **81**(11), 4414–4421 (2009).
- ⁸¹R. Ziv, Y. Steinhardt, G. Pelled, D. Gazit, and B. Rubinsky, *Biomed. Microdevices* **11**(1), 95 (2009).
- ⁸²K.-Y. Lu, A. M. Wo, Y.-J. Lo, K.-C. Chen, C.-M. Lin, and C.-R. Yang, *Biosens. Bioelectron.* **22**(4), 568–574 (2006).
- ⁸³N. Cordero, J. West, and H. Berney, *Microelectron. J.* **34**(12), 1137–1142 (2003).
- ⁸⁴L. C. Waters, S. C. Jacobson, N. Kroutchinina, J. Khandurina, R. S. Foote, and J. M. Ramsey, *Anal. Chem.* **70**(1), 158–162 (1998).
- ⁸⁵C. Ke, A.-M. Kelleher, H. Berney, M. Sheehan, and A. Mathewson, *Sens. Actuators B* **120**(2), 538–544 (2007).
- ⁸⁶S.-K. Baek, J. Min, and J.-H. Park, *Lab. Chip* **10**(7), 909–917 (2010).
- ⁸⁷J. P. Kutter, S. C. Jacobson, and J. M. Ramsey, *J. Microcolumn Sep.* **12**(2), 93–97 (2000).
- ⁸⁸G. R. Duarte, C. W. Price, J. L. Littlewood, D. M. Haverstick, J. P. Ferrance, E. Carrilho, and J. P. Landers, *Analyst* **135**(3), 531–537 (2010).
- ⁸⁹N. C. Cady, S. Stelick, M. V. Kunnavakkam, and C. A. Batt, *Sens. Actuators B* **107**(1), 332–341 (2005).
- ⁹⁰H. Tian, A. F. Hühmer, and J. P. Landers, *Anal. Biochem.* **283**(2), 175–191 (2000).
- ⁹¹M. C. Breadmore, K. A. Wolfe, I. G. Arcibal, W. K. Leung, D. Dickson, B. C. Giordano, M. E. Power, J. P. Ferrance, S. H. Feldman, and P. M. Norris, *Anal. Chem.* **75**(8), 1880–1886 (2003).
- ⁹²C. R. Reedy, C. W. Price, J. Sniegowski, J. P. Ferrance, M. Begley, and J. P. Landers, *Lab. Chip* **11**(9), 1603–1611 (2011).
- ⁹³M. Karle, J. Miwa, G. Czilwik, V. Auwärter, G. Roth, R. Zengerle, and F. von Stetten, *Lab. Chip* **10**(23), 3284–3290 (2010).
- ⁹⁴P. Wilding, M. A. Shoffner, and L. J. Kricka, *Clin. Chem.* **40**(9), 1815–1818 (1994).
- ⁹⁵E. A. Oblath, W. H. Henley, J. P. Alarie, and J. M. Ramsey, *Lab. Chip* **13**(7), 1325–1332 (2013).
- ⁹⁶M. U. Kopp, A. J. De Mello, and A. Manz, *Science* **280**(5366), 1046–1048 (1998).
- ⁹⁷J. Liu, M. Enzelberger, and S. Quake, *Electrophoresis* **23**(10), 1531–1536 (2002).
- ⁹⁸D. Moschou, N. Vourdas, G. Kokkoris, G. Papadakis, J. Parthenios, S. Chatzandroulis, and A. Tserepi, *Sens. Actuators B* **199**, 470–478 (2014).
- ⁹⁹Y. Zhang and H.-R. Jiang, *Anal. Chim. Acta* **914**, 7–16 (2016).
- ¹⁰⁰Y. Schaeferli, R. C. Wootton, T. Robinson, V. Stein, C. Dunsby, M. A. Neil, P. M. French, A. J. DeMello, C. Abell, and F. Hollfelder, *Anal. Chem.* **81**(1), 302–306 (2008).
- ¹⁰¹J. Wang, *Electroanalysis* **17**(13), 1133–1140 (2005).
- ¹⁰²B. S. Ferguson, S. F. Buchsbaum, J. S. Swensen, K. Hsieh, X. Lou, and H. T. Soh, *Anal. Chem.* **81**(15), 6503–6508 (2009).
- ¹⁰³L. Wang and P. C. Li, *Anal. Chim. Acta* **687**(1), 12–27 (2011).
- ¹⁰⁴A. Kotani, M. A. Witek, J. K. Osiri, H. Wang, R. Sinville, H. Pincas, F. Barany, and S. A. Soper, *Anal. Methods* **4**(1), 58–64 (2012).
- ¹⁰⁵H. Zhang, J. Song, H. Ren, Z. Xu, X. Wang, L. Shan, and J. Fang, *PLoS ONE* **8**(1), e54510 (2013).
- ¹⁰⁶T. H. Fang, N. Ramalingam, D. Xian-Dui, T. S. Ngien, Z. Xianting, A. T. L. Kuan, E. Y. P. Huat, and G. Hai-Qing, *Biosens. Bioelectron.* **24**(7), 2131–2136 (2009).
- ¹⁰⁷R. Y. Lai, E. T. Lagally, S.-H. Lee, H. Soh, K. W. Plaxco, and A. J. Heeger, *Proc. Natl. Acad. Sci. U.S.A.* **103**(11), 4017–4021 (2006).
- ¹⁰⁸M. Labib, S. M. Ghobadloo, N. Khan, D. M. Kolpashchikov, and M. V. Berezovski, *Anal. Chem.* **85**(20), 9422–9427 (2013).
- ¹⁰⁹M. N. Islam, V. Gopalan, M. H. Haque, M. K. Masud, M. S. Al Hossain, Y. Yamauchi, N.-T. Nguyen, A. K.-Y. Lam, and M. J. Shiddiky, *Biosens. Bioelectron.* **98**, 227–233 (2017).

- ¹¹⁰J. A. Sánchez, O. Henry, H. Joda, B. W. Solnestam, L. Kvastad, E. Johansson, P. Akan, J. Lundeberg, N. Lladach, and D. Ramakrishnan, *Biosens. Bioelectron.* **82**, 224–232 (2016).
- ¹¹¹T. Okamoto, T. Suzuki, and N. Yamamoto, *Nat. Biotechnol.* **18**(4), 438–441 (2000).
- ¹¹²A. S. Ishkanian, C. A. Malloff, S. K. Watson, B. Chi, B. P. Coe, A. Snijders, D. G. Albertson, D. Pinkel, M. A. Marra, and V. Ling, *Nat. Genet.* **36**(3), 299 (2004).
- ¹¹³D. Martins, X. Wei, R. Levicky, and Y.-A. Song, *Anal. Chem.* **88**(7), 3539–3547 (2016).
- ¹¹⁴T. M. Yen, T. Zhang, P.-W. Chen, T.-H. Ku, Y.-J. Chiu, I. Lian, and Y.-H. Lo, *ACS Nano* **9**(11), 10655–10663 (2015).
- ¹¹⁵P. Liu and R. A. Mathies, *Trends Biotechnol.* **27**(10), 572–581 (2009).
- ¹¹⁶S. Zheng, H. Lin, J.-Q. Liu, M. Balic, R. Datar, R. J. Cote, and Y.-C. Tai, *J. Chromatogr. A* **1162**(2), 154–161 (2007).
- ¹¹⁷T. Geng, N. Bao, N. Sriranganathanw, L. Li, and C. Lu, *Anal. Chem.* **84**(21), 9632–9639 (2012).
- ¹¹⁸S. K. Jha, R. Chand, D. Han, Y.-C. Jang, G.-S. Ra, J. S. Kim, B.-H. Nahm, and Y.-S. Kim, *Lab. Chip* **12**(21), 4455–4464 (2012).
- ¹¹⁹D. Le Roux, B. E. Root, J. A. Hickey, O. N. Scott, A. Tsuei, J. Li, D. J. Saul, L. Chassagne, J. P. Landers, and P. De Mazancourt, *Lab. Chip* **14**(22), 4415–4425 (2014).
- ¹²⁰D. Summerer, D. Hevroni, A. Jain, O. Oldenburger, J. Parker, A. Caruso, C. F. Stähler, P. F. Stähler, and M. Beier, *New Biotechnol.* **27**(2), 149–155 (2010).
- ¹²¹E. Roy, G. Stewart, M. Mounier, L. Malic, R. Peytavi, L. Clime, M. Madou, M. Bossinot, M. G. Bergeron, and T. Veres, *Lab. Chip* **15**(2), 406–416 (2015).
- ¹²²Z. Slouka, S. Senapati, S. Shah, R. Lawler, Z. Shi, M. S. Stack, and H.-C. Chang, *Talanta* **145**, 35–42 (2015).
- ¹²³R. M. Mohamadi, I. Ivanov, J. Stojcic, R. K. Nam, E. H. Sargent, and S. O. Kelley, *Anal. Chem.* **87**(12), 6258–6264 (2015).
- ¹²⁴D. D. Taylor and C. Gercel-Taylor, *Gynecol. Oncol.* **110**(1), 13–21 (2008).
- ¹²⁵M. Wu, Y. Ouyang, Z. Wang, R. Zhang, P.-H. Huang, C. Chen, H. Li, P. Li, D. Quinn, M. Dao, S. Suresh, Y. Sadovsky, and T. J. Huang, *Proc. Natl. Acad. Sci.* **114**(40), 10584–10589 (2017).
- ¹²⁶A. Liga, A. Vliegenthart, W. Oosthuyzen, J. Dear, and M. Kersaudy-Kerhoas, *Lab. Chip* **15**(11), 2388–2394 (2015).
- ¹²⁷S. S. Kanwar, C. J. Dunlay, D. M. Simeone, and S. Negrath, *Lab. Chip* **14**(11), 1891–1900 (2014).
- ¹²⁸B. Hong and Y. Zu, *Theranostics* **3**(6), 377 (2013).