Microfluidics cell sample preparation for analysis: Advances in efficient cell enrichment and precise single cell capture

Liang Huang,[1] Shengtai Bian,[2] Yinuo Cheng,[1] Guanya Shi,[3] Peng Liu,[2] Xiongying Ye,[1] and Wenhui Wang[1,a)]
[1]*State Key Laboratory of Precision Measurement Technology and Instrument, Department of Precision Instrument, Tsinghua University, Beijing, China*
[2]*Department of Biomedical Engineering, Tsinghua University, Beijing, China*
[3]*Department of Automotive Engineering, Tsinghua University, Beijing, China*

(Received 17 October 2016; accepted 24 January 2017; published online 6 February 2017)

Single cell analysis has received increasing attention recently in both academia and clinics, and there is an urgent need for effective upstream cell sample preparation. Two extremely challenging tasks in cell sample preparation—high-efficiency cell enrichment and precise single cell capture—have now entered into an era full of exciting technological advances, which are mostly enabled by microfluidics. In this review, we summarize the category of technologies that provide new solutions and creative insights into the two tasks of cell manipulation, with a focus on the latest development in the recent five years by highlighting the representative works. By doing so, we aim both to outline the framework and to showcase example applications of each task. In most cases for cell enrichment, we take circulating tumor cells (CTCs) as the target cells because of their research and clinical importance in cancer. For single cell capture, we review related technologies for many kinds of target cells because the technologies are supposed to be more universal to all cells rather than CTCs. Most of the mentioned technologies can be used for both cell enrichment and precise single cell capture. Each technology has its own advantages and specific challenges, which provide opportunities for researchers in their own area. Overall, these technologies have shown great promise and now evolve into real clinical applications. *Published by AIP Publishing.* [http://dx.doi.org/10.1063/1.4975666]

I. INTRODUCTION

Cell analyses provide direct answers to basic and great scientific questions in life sciences. Advances in cell analysis techniques have been more vigorous than ever before in order to meet the high expectations of accuracy, throughput, efficiency, precision, automation, resolution, and speed. In line with the development of "-omics" (e.g., genomics and proteomics), cell analysis techniques have been developed rapidly and widely used in applications such as cancer study, tissue and organ regeneration, drug screening, gene therapies, etc.[1–5] In the early period of cell analysis, cells in a population were assumed homogeneous. However, this assumption was found invalid as even individual cells with the same genotype show heterogeneity.[6,7] Thus, the goal of single cell analysis is to analyze transcription, translation, regulatory, and signaling events within individual cells at the molecular level.[8] All the molecular level activity, gene expression and signal transduction of cells have significant differences, thus necessitating single cell analysis and calling for new technologies. In the context of precision medicine, single cell analysis has received strong support from major governments. For example, the US NIH initiated a program specialized in single cell analysis in the late 2012 with the investment of USD

a)Author to whom correspondence should be addressed. Electronic mail: wwh@mail.tsinghua.edu.cn

90 million over 5-year period, and some great achievements have been reported in the literature.[9–12]

Single cell analysis could reveal genomics and proteomics that are hidden in bulk measurements, which require cell samples to be well-prepared before the target cells could be actually analyzed (Fig. 1(a)).[13,14] High-efficiency cell enrichment and precise single cell capture with extreme challenges have now entered into an era full of exciting technological advances, thanks to microfluidics. Microfluidic systems have been powerful tools for the study of single cells with the following main advantages,[15–17] including: (1) Small volume sample and reagent consumption: The sizes of channels and structures in microfluidic chips are usually at the micro level, so only several milliliters (even microliters) of sample and reagent are needed. This well-suited for rare cells, e.g., circulating tumor cells (CTCs). (2) Well-defined fluidic characteristics: The solution in microchannels can easily form laminar flow and droplets[18–20] to facilitate single cell manipulation and analysis. (3) Easy combination with multiple technologies: Microfluidics can form a powerful platform by integrating multiple biochemical and physical methods, such as optical tweezes,[21,22] magnetics[23,24] electrics[25,26] and acoustic waves.[27,28] (4) Large scale and high throughput: Microfluidic chips can normally be scaled up to large-array units for single cell manipulation and analysis. Tens of thousands of cell processing units per chip are possible and also multiple chips can be stacked to increase throughput to allow ml/min scale flow rate, such that one tube of 10 ml bloodstream could be processed at 10-min time scale.

Single cell analysis often focuses on specific cell lines and primary cells due to availability and clinical importance. In this paper, we choose circulating tumor cells (CTCs) as the cell of interest to review the high-efficiency cell enrichment technologies due to the following two major reasons: (1) CTCs in patients have been an interesting topic of scientific investigations, in view of their clinical importance for the metastatic process of carcinomas and clinical diagnosis.[29–34] (2) CTCs are extremely rare in the peripheral blood that also contains red cells, white cells, and blood platelets (about one CTC out of 1×10^9 blood cells). The CTCs become so important and thus attract attention, indicated by the chronologically increasing number of research papers (Fig. 1(b)). On the other hand, when reviewing technologies for precise single cell capture, we deliberately do not differentiate the cell type because cell samples at this stage would presumably be quite pure and homogeneous.

Cell enrichment is essentially to screen one target cell type out of the cell sample mixing multiple cell types, including body fluid like blood. Ideally, target cells should have distinctive features (such as generally biophysical or biochemical parameters) to be differentiated from other cells. For example, in CTCs, their physical size, mechanical stiffness, electrical properties, and surface binding to particular antibodies have been proven specific somehow for enrichment.[35,36] In order to achieve the desired specifications such as throughput, purity, and enrichment factor, a range of device structures can be solely or in combination designed in one chip. The outcome of ideal cell enrichment is relatively pure and high-density target cells in population. Individual cells need to be further singled out from pure and high-density target cells for downstream tasks, e.g., either

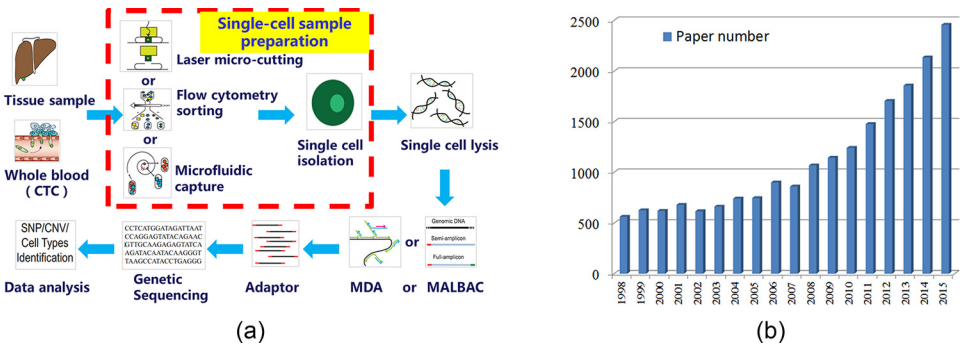


FIG. 1. Single cell analysis procedure and number of research papers about CTCs. (a) Schematic of single cell analysis procedure.[13,14] (b) The number of research papers about CTCs increases sharply from 1998 to 2015.

on-site study (e.g., long-term culture and imaging) or off-site manipulation (e.g., transfer and lysis) and analysis (e.g., polymerase chain reaction (PCR), DNA/RNA sequencing or profiling).

Regardless of the downstream tasks, single cell capture plays a critical role and the challenges lie in high efficiency, large scale, flexibility, and combination with cell translocation function. However, single cell manipulation is not straightforward. Therefore, more efficient and operational strategies able to control each cell individually become a hot topic gradually. Extensive research has been reported in order to capture single cells using innovative techniques, such as mechanics, magnetics, optics, electrics, acoustics and droplets. Each of these methods is associated with some advantages and disadvantages that make them preferable in certain circumstances. From the practical perspective, cell sample preparation is better integrated as one stand-alone module, which could be used like a Fluidigm C1 cartridge.[37] However, such product-oriented design concept is more deployed in companies rather than in research laboratories and is not the focus of this review.

In this review, we summarize the category of technologies that provide new solutions and creative insights into the above-mentioned two tasks of cell manipulation, with a focus on the latest development in the recent five years by highlighting the representative works. By doing so, we aim both to outline the framework and to showcase example applications of each technology. During the preparation of this review, we found a lot of excellent review papers in related topics and have compiled them in Table I for the benefit of our readers. In the meantime, we minimize the overlapping of our review with existing literature by covering only the two key cell sample preparation tasks and using the latest and different works as the technology advancement highlight.

II. SINGLE CELL ENRICHMENT

CTCs are extremely rare in the peripheral circulation. The concentration is about only a single CTC per billion normal blood cells in the patients with advanced cancer. Therefore, the enrichment of CTCs is essential for further analysis. Up to date, cell enrichment techniques mainly use physical and biological features to separate CTCs from the other bloodstream cells. Correspondingly, the single cell enrichment techniques are divided into two categories: physical techniques and biological techniques. The former includes filtration, hydrodynamics, electric charge, optical tweezers and acoustic wave, while the latter mainly includes affinity reaction and immunomagnetics.

A. Physical techniques

1. Filtration

The size-dependent filter-based microfluidic devices exhibit numerous advantages, such as high labeling efficiency, short detection time, high detection sensitivity, and high reproducibility, thanks to the simple and robust experimental procedures. Filtration provides the size-based separation of CTCs on the premise that they are larger than normal white and red blood cells. On average, CTCs are larger than blood cells. For example, most cancer cells measure more than 15 μm in size, whereas most peripheral blood leukocytes measure from 8 to 11 μm. Using size information, filtration method is fast, simple, straightforward, and reliable.[49–53] Recently, a filtration chip with lateral flow was combined with the vertical flow into the filter to capture the CTCs gently (Fig. 2(a)).[54] CTCs experienced weak shear flow owing to the lateral flow and traveled alongside the filter channel until finally being captured. The vertical flow in the filter held the captured cells tightly and served as an exit for uncaptured hematological cells. The chip can obtain a high capture efficiency (95%) and purity (99%), minimizing any damage to CTCs.

However, most microfiltration-based cell separation microfluidic chips still suffer from low-throughput and membrane clogging. To overcome the issues, Cheng *et al*. reported on a bubble-free and clogging-free microfluidic particle separation platform with high throughput (Fig. 2(b)).[55] The platform features with an integrated bidirectional micropump, a hydrophilic microporous filtration membrane and a hydrophobic porous degassing membrane. The bidirectional micropump enables

TABLE I. Recent reviews about cell analysis.

Title, year	Main content
"Microfluidic techniques for high throughput single cell analysis,"[38] 2016	Techniques based on both active and passive manipulation, capable of discriminating between single cell phenotypes for sorting, diagnostic or prognostic applications
"Single cells in confined volumes: microchambers and microdroplets,"[39] 2016	Two complementary approaches: (i) the isolation of cells in small chambers defined by microchannels and integrated valves and (ii) the encapsulation of cells in microdroplets
"Single-cell microfluidics: opportunity for bioprocess development,"[40] 2014	Microfluidics systems in five biotechnological processes: (i) growth and morphology, (ii) population heterogeneity, (iii) process characterization, (iv) dynamic environments and gradients, and (v) strain characterization
"Microfluidics for cell-based high throughput screening platforms—A review,"[41] 2016	The application of microfluidics in cell based high throughput screening, using the perfusion flow mode, the droplet mode, and the microarray mode
"Microfluidic sample preparation for single cell analysis,"[42] 2016	Sample preparation steps that may be necessary for characterizing single cells; tissue dissociation into cell suspensions; sorting heterogeneous cell populations into homogeneous populations, isolating, and lysing single cells
"Microfluidics for manipulating cells,"[43] 2013	Four subfields among the diverse achievements in microfluidics: cell patterning, microenvironment patterning, cell screening and single-cell analysis
"Microfluidics in systems biology—hype or truly useful,"[44] 2016	Applications where microfluidics can enhance experimental sensitivity and throughput, particularly in single-cell analyses and analyses on multi-cellular or complex biological entities
"Microtools for single-cell analysis in biopharmaceutical development and manufacturing,"[45] 2013	Microtools for single-cell analysis, such as array wells, microfluidic traps, valves and droplet
"Advances of lab-on-a-chip in isolation, detection and post-processing of circulating tumor cells,"[46] 2013	How miniaturisation strategies together with nanotechnologies have been used to advance LOC for capturing, separating, enriching and detecting different CTCs efficiently
"Droplet microfluidics for microbiology: techniques, applications and challenges,"[47] 2016	Droplet microfluidics for microbiology (techniques, applications and challenges)
"Droplet microfluidics in (bio)chemical analysis,"[48] 2015	Advances in droplet formation and manipulation technology and chemical analysis applications with the focus on new developments

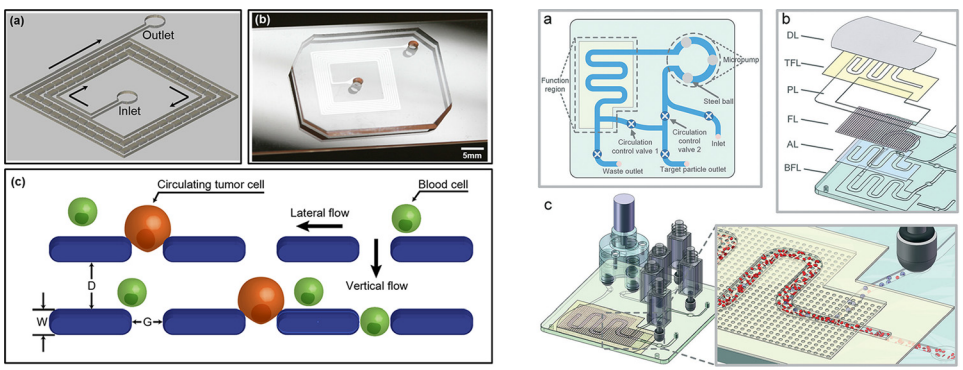


FIG. 2. Size-based separation of CTCs. (a) Schematic drawing and photographic image of the microfluidic lateral flow filtration.[54] Reproduced with permission from Lee *et al.*, J. Chromatogr. A **1377**, 100 (2015). Copyright 2015 Elsevier. (b) The bubble-free particle separation chip.[55] Reproduced with permission from Cheng *et al.*, Lab Chip **16**, 4517 (2016). Copyright 2016 Royal Society of Chemistry.

the fluid to flow back and forth repeatedly, which flush the filtration membrane and clear the filtration micropores for further filtration, and to flow forward to implement multifiltration. The hydrophobic porous membrane on top of the separation channel removes air bubbles forming in the separation channel, improving the separation efficiency and operational reliability. White blood cells are effectively separated from the whole blood with 396-fold enrichment ratio and 70.6% recovery rate at throughput of 39.1 μl/min after 8 cycles.

Although filter-based microfluidic devices exhibit numerous advantages, the applications for cell-based assay are limited by their poor selectivity. The constantly stranded chip blockage of cells may occur on the filter structure. Removing the stranded cells from the filter on the premise of not damaging the cell viability is also a more difficult problem, which should be taken into account in designing such a device.

2. Hydrodynamics

a. Inertial force. Inertial force-based cell separation has been demonstrated using flows in curvilinear microchannels. As the technique is membrane-free that eliminates issues arising from membrane fouling, it can be performed in a continuous flow mode allowing the processing of large sample volumes in a short time. Without requiring an external force field, inertial force-based devices are easy to fabricate and can be applied to any particle type ranging from biological samples such as cells to micrometer-sized colloidal particles. When particles flow within a spiral microchannel, buoyant particles under the influence of inertial lift forces arising from the parabolic nature of the laminar velocity profile migrate across the streamlines to an equilibrium position. Then, the particles will move away from the channel center towards the channel walls. The particles experience a drag force introduced by the Dean vortices along the Dean flow arising due to the curvilinear geometry at the same time. The combination of inertial and Dean forces reduces the equilibrium positions to a single position at the inner microchannel wall within the channel width, inducing continuous inertial focusing. Since both forces are functions of the particle size, the particles of different size occupy distinct lateral positions near the channel wall and exhibit different degrees of focusing, allowing size-based separation.[56–60]

The smaller blood cells, including red blood cells and leukocytes, migrate along the Dean vortices toward the inner wall and then back to the outer wall again, while the larger CTCs experience additional strong inertial lift forces and focus along the microchannel inner wall. The advantage of the hydrodynamic method is that it offers a quick, simple and label-free way to isolate CTCs. But the drawback is its poor sensitivity due to the loss of some CTCs migrating to the plasma layer, cell-to-cell interactions, or the formation of CTC aggregates settling to the bottom of the gradient. Warkiani *et al.* reported a label-free spiral microfluidic device to allow size-based isolation of viable CTCs using the hydrodynamic forces that are present in curvilinear microchannels (Fig. 3). The spiral system enables us to achieve $\geq$85% recovery of spiked cells across multiple cancer cell lines and 99.99% depletion of white blood cells in whole blood.[16]

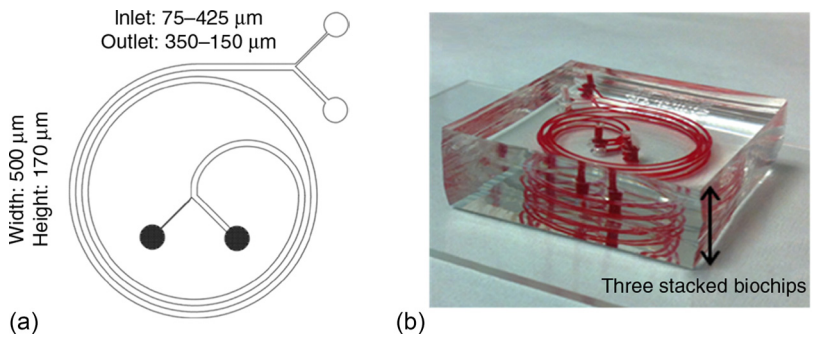


FIG. 3. A label-free spiral microfluidic device to allow size-based isolation of viable CTCs using hydrodynamic forces. (a) The device design consists of a two-loop spiral microchannel with two inlets and two outlets. (b) Photograph of three stacked biochips.[16] Reproduced with permission from Warkiani *et al.*, Nat. Protoc. **11**, 134 (2016). Copyright 2016 Nature Publishing Group.

b. Deterministic lateral displacement (DLD). DLD is another cell separation method using hydrodynamic force and channel geometric force balance, including fluidic resistance from the arrayed pillars. Compared to the traditional filter methods, DLD methods have significantly higher throughput and more distinct advantages, such as label-free and clogging-free, and maintain high cell viability after processing. The main mechanism of DLD is shown in Fig. 4(a). Different drag forces and DLD forces are applied to the cells of different diameter, as they move along the streamlines of the flow. Larger size cells will move laterally toward to the side of channel; smaller particles will flow along the original trajectory.[61–65]

DLD arrays allow continuous cancer cell enrichment from peripheral blood. However, the cancer cell purity was still very low after enrichment. Liu *et al.* introduced an integrated microfluidic system for continuous high throughput cancer cell capture with high yield and purity. The system combining the microfluidic DLD array and affinity-based technique can enrich and capture CTCs fast and effectively (Fig. 4(b)).[66] Using this device to isolate breast cancer cells from the spiked blood samples achieved an enrichment factor of 1500× and a high processing throughput of 9.6 ml/min with 90% capture yield and more than 50% capture purity at the cell density of 10^2 cells/ml.

Hydrodynamic enrichment methods are geometry-sensitive in which the geometry of the device is critical. On good side, normally the geometry can be straightforwardly finalized, so that the design and fabrication do not cause big trouble. However, the cells that need to be separated should be of different size or shape than the other cells, which is the prerequisite for this technique to be applied.

3. Dielectrophoresis

Dielectrophoresis (DEP) has been highly regarded as a useful tool for the separation and manipulation of cells in microsystems due to the merits of label-free, low-damage, high-efficiency, and easy-operation.[67–69] DEP separation and enrichment of CTCs features high specificity and viability. DEP is the electrokinetic motion that occurs when a polarizable particle is placed in the non-uniform electric fields. Because most biological cells have dielectric characteristics in an external electric field, cells in suspension can be controlled by DEP force or torque.[70–72] Cells can be stimulated to travel to the region with a strong electric field by a positive DEP force in the non-uniform electric field, or conversely, to the area with a weak electric field by a negative DEP force.

DEP offers flexible control schemes by using the electric field parameters (such as amplitude and frequency) to manipulate cells. DEP-based continuous cell sorting has been successfully exploited to distinguish bacteria, mammalian cells, blood cells, and cancer cells. Song *et al.* presented a continuous-flow microfluidic device for sorting stem cells and their differentiation progenies (Fig. 5(a)).[73] The principle of the device is based on the accumulation of multiple DEP forces to deflect cells laterally in conjunction with the alternating on/off electric field

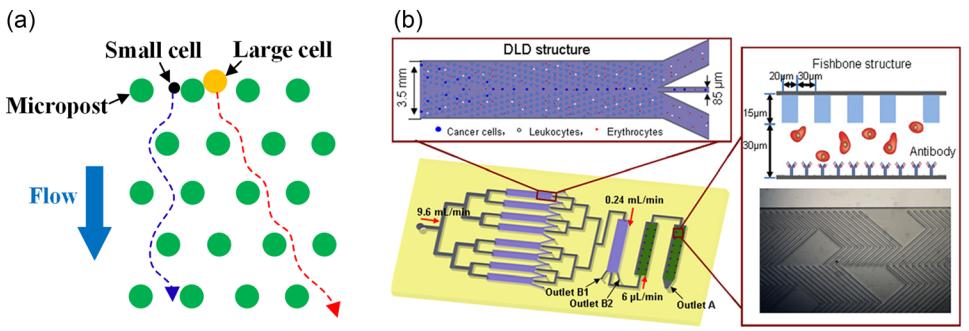


FIG. 4. Cell enrichment via DLD. (a) The main mechanism of DLD. (b) An integrated microfluidic system for fast and efficient CTCs enrichment and capture.[66] Reproduced with permission from Liu *et al.*, Biosen. Bioelectron. **47**, 113 (2013). Copyright 2013 Elsevier.

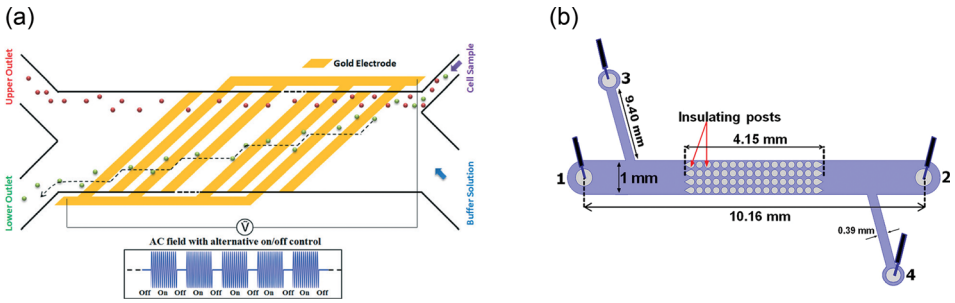


FIG. 5. (a) A continuous-flow microfluidic device for sorting stem cells.[73] Reproduced with permission from Song *et al.*, Lab Chip **15**, 1320 (2015). Copyright 2015 Royal Society of Chemistry. (b) A microfluidic chip based on insulated DEP for sorting cells.[79] Reproduced with permission from LaLonde *et al.*, Biomicrofluidics **9**, 64113 (2015). Copyright 2015 AIP Publishing.

to manipulate the cell. Collection efficiencies up to 92% and 67% for stem cells and osteoblasts along with purity up to 84% and 87%, respectively, were achieved.

The insulators for producing DEP effects were discovered later and also used in microfluidics for sorting cells.[74–78] Insulated pillars in microchannels change the distribution of electric fields and form strong DEP forces at the edge of insulated structure to capture cells. LaLonde *et al.* presented an insulator-based dielectrophoresis (iDEP) device for the detection and stable capture of low abundant polystyrene particles and yeast cells, as show in Fig. 5(b). The device demonstrated successful and stable capture and enrichment of rare particles and cells (trapping efficiencies over 99%), where particles stably remained trapped for up to 4 min.[79] Usually, the insulator-based systems are simpler devices and made from a single substrate, so fabrication processes are low-cost and convenient. However, very high voltage is required to generate enough DEP force.

Although DEP can be conveniently used for the continuous separation of cells, it still has some limitations. (a) If the electrical characteristics of target cells and non-target cells are nearly the same, it is hard to distinguish and separate them. (b) Cell will be damaged or even broken when it directly contacts the electrodes. (c) The Joule heating will cause fluid convection that can reduce the DEP separation efficiency. For more on DEP, we refer the reader to an excellent review.[80]

4. Optical tweezers

Optical tweezers are a single beam optical gradient trap that permits non-invasive and non-destructive manipulation of cells and is useful for sorting and separation of cells. They are especially advantageous in the manipulation of single cells since properly chosen forces are strong enough. Spatial light distributions in intensity and shape can be switched rapidly. Furthermore, systems based on optical tweezers do not need any electrodes. When a laser beam is focused on a particle or cell, it forms a three dimensional optical potential well that can induce optical pressure to capture particle. The captured particles or cells can be transported for sorting[81–84] or patterning[85,86] by controlling the track of optical tweezers.

The sorting methods based on optical tweezers can be classified into passive and active modes. Passive sorting methods use a specifically designed static light distribution, typically an optical lattice with a designed grating width to move cells in a microfluidic channel. The cells must exhibit differences in refractive index, size or shape, which could induce different optical forces in light field. The drawback of passive methods is that sorting depends on differences of the intrinsic cell properties. Active sorting methods are able to sort cells based on the label characteristics: for example, fluorescence signals and electric markers. The target cells after being identified can be sorted out by collecting the cells in an isolated outlet of the microfluidic system. Although active sorting methods are reliable, a feedback control system incorporating the label detecting sensor is essential for automation. If feedback is achieved manually, it will be very time consuming to isolate single cells from cell population.

For active sorting methods, to shorten the processing time, Landenberger *et al.* presented an optical particle sorter that uses rapidly steerable optical traps to displace cells within a laminar flow inside a microchannel (Fig. 6(a)).[83] Cells not grabbed by the optical tweezers stream into a different reservoir than those actively displaced to a parallel streamline. The system is preferably suitable for small populations of a few hundred to thousand cells. The speed of sorting cells can reach 1.4 cells/s with the sorting accuracy of 89%.

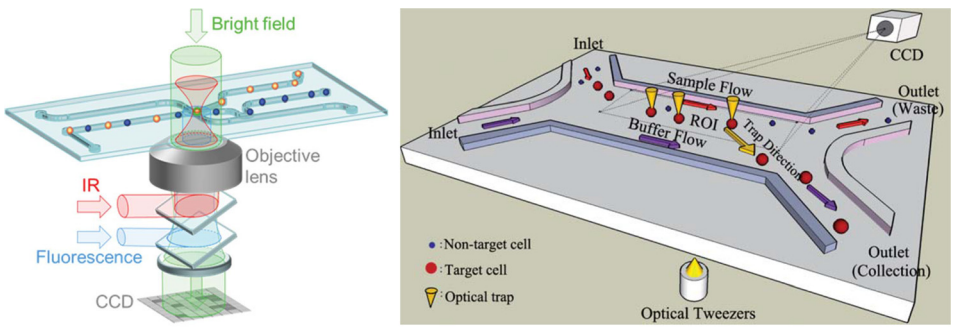
When only one type of cell is labeled in optical tweezers, cell enrichment suffers from poor accuracy because only one feature was not sufficient. Wang *et al.* introduced a generic single cell manipulation tool for sorting small cell populations (Fig. 6(b)).[87] The so-called cell sorter was designed based on dynamic fluid and light pattern, which are capable of recognizing multiple features of cells, e.g., cell size and fluorescence label. A CCD camera was used to capture and recognize target cells, and the images were analyzed to guide optical tweezers for handling and moving the target cells to the desired outlet. The advantages of the sorter are its high recovery rate and purity in sorting the small cell population. It can achieve 96% purity in the sorting of yeast cells and 90% purity for human embryonic stem cells.

However, optical tweezer-based cell enrichment methods are inherently limited to manipulate a small number of particles in real-time due to their complex and expensive constructions. The low-throughput for single optical trap and complexity for multiple optical traps prevent the technique from being used widely for large-scale cell samples to be processed, but they are competitive tools for the precise manipulation of a small number of cells.

5. Acoustics

Surface acoustic wave (SAW)-based sorting methods have been used widely with the advantages of non-contact manipulation, low cost, high controllability, and high biocompatibility.[88–90] Acoustic separation is often achieved by establishing a standing acoustic field within a flow channel. When a standing acoustic field is present in a fluid medium, particles (or cells) populating the fluid will be pushed toward regions with a minimal acoustic radiation pressure. Particles with different sizes and physical properties will experience different acoustic radiation forces and will take different time to migrate to the pressure nodes, thus providing clear identifiers for separation. In SAW, the shape of acoustic fields can be easily tuned by choosing an appropriate interdigital transducer (IDT) pattern and location. The interaction between SAW and fluid medium can result in acoustic streaming that moves cells along the fluid circulation.[91–95]

Although acoustic cell enrichment has successfully separated the cancer cells of cell lines from WBCs, it has not been applied to the separation of rare CTCs from the clinical samples.



(a) (b)

FIG. 6. (a) An optical particle sorter that uses rapidly steerable optical traps to displace cells within a laminar flow inside a microchannel.[83] Reproduced with permission from Landenberger *et al.*, Lab Chip **12**, 3177 (2012). Copyright 2012 Royal Society of Chemistry. (b) A single cell manipulation tool based on dynamic fluid and a dynamic light pattern for sorting small cell populations.[87] Reproduced with permission from Wang *et al.*, Lab Chip **11**, 3656 (2011). Copyright 2011 Royal Society of Chemistry.

It is mainly due to insufficient throughput. To increase the throughput, Li *et al.* presented an acoustic-based microfluidic device that is capable of high-throughput separation of CTCs from peripheral blood samples (Fig. 7(a)).[96] The method uses tilted-angle standing surface acoustic waves and successfully separated low concentrations (~100 cells/ml) of a variety of cancer cells of the cell culture lines from WBCs with a recovery rate better than 83%.

In the case of standing SAW, the maximum translation displacement of cell/particle is less than one quarter of the acoustic wave length, and the minimum beam width is on the order of the acoustic wavelength. Hence, the smallest wavelength can be used to achieve the translation distance in single particle level. Collins *et al.* demonstrated the use of highly localized acoustic fields generated by focused SAW for single particle level displacement, where deterministic sorting is made possible using a focused beam with a width of only 25 μm (Fig. 7(b)).[97] Focused SAW was generated by a high-frequency (386 MHz), 10 μm wavelength set of focused interdigital transducers (FIDTs) on a piezoelectric lithium niobate substrate. Objects with a diameter down to 2 μm can be translated on-demand using this frequency, and varying pulse lengths can also be used to form time-varying particle concentrations. The highly focused SAW is ideally suitable for deterministic sorting and microfluidic manipulation, holding potential for small specimens down to the scale of bacteria.

Acoustic cell enrichment offers a means of separating cells on the basis of their size and physical properties in a label-free, contactless, and biocompatible manner. The separation sensitivity and efficiency of currently available acoustic based approaches, however, are limited, thereby restricting their widespread application in research and health diagnostics.

B. Biological techniques

1. Affinity reaction

Affinity-based cell enrichment methods are often used when cell-secreting biomarkers have different affinities with specific antibody coated on the surface of microchannels or microstructures. Using the specificity of antigen-antibody reaction, these biological methods result in very high-accuracy enrichment. There are two types of affinity cell enrichment methods (positive and negative enrichment). Positive enrichment isolates the target cells using the interactions between target cell surface antigens and antibodies. In contrast, negative enrichment specifically eliminates the non-target cells and collects all the rare cells, regardless of their immune–cytochemical expression level. Anti-epithelial cell adhesion molecules (EpCAM) are one of the typical biomarkers for CTCs. Anti-EpCAM antibody coating has been demonstrated to have an outstanding cell capture efficiency in both static and dynamic systems.[98–101] In the case of

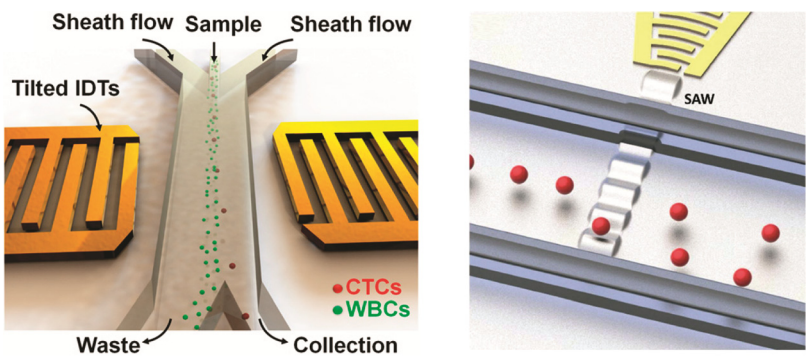


FIG. 7. Acoustic methods for cells enrichment. (a) Illustration of SAW-based cell separation and enrichment.[96] Reproduced with permission from Li *et al.*, Proc. Natl. Acad. Sci. U.S.A. **112**, 4970 (2015). Copyright 2015 National Academy of Sciences. (b) A highly focused SAW is used to sort at the single-particle level, with the width of the focused SAW on the order of tens of μm.[97] Reproduced with permission from Collins *et al.*, Lab Chip **16**, 471 (2016). Copyright 2016 Royal Society of Chemistry.

CTCs, most positive enrichment is grounded on the EpCAM antibodies. For example, He *et al.* proposed a biocompatible and surface roughness controllable nano-film which is composed of TiO_2 nanoparticles for highly efficient CTC capture and *in-situ* identification by immunocytochemistry (Fig. 8(a)).[102] The nano-film was coated with an anti-EpCAM antibody for the specific capturing of EpCAM positive cancer cells.

However, positive enrichment has two major significant limitations in practice. One limitation is the lack of information about the phenotype of the target cell. Using a specific antibody to capture CTCs may lose a large fraction of the target cells because not all CTCs express EpCAM at the same levels under any physiological conditions. The other one is that the isolated cells can be modified by antibody attachment, which is not desirable for downstream studies, such as cellular and molecular analysis.

Compared to positive enrichment, negative enrichment without considering the immune–cytochemical expression level of target cells is more suitable for rare cell isolation and further analysis. Hyun *et al.* developed a geometrically activated surface interaction chip with an asymmetric herringbone structure designed to generate enhanced mixing flows, increasing the surface interaction between the non-target cells and the channel surface.[103] The CD45 antibodies were immobilized inside the channel to capture leukocytes and release CTCs to the outlet (Fig. 8(b)). The herringbone structure was patterned on the channel to produce transverse flow that facilitates effective contact between antigens on the cells and antibodies on the channel surface. The chip significantly improved the capture efficiency to 98.94% and enrichment yield to 130.94-fold.

Most affinity reaction based CTC enrichment assays rely on the expression of the cell surface biomarkers from either the target cell (e.g., EpCAM of CTCs in positive enrichment) or the non-target cell (CD45 of white blood cells in negative enrichment). The big challenge is that the biomarker expression levels are not certain in the sample medium for different cancers, different stages of the same cancer, and different persons. It is highly desirable to identify more biomarkers than the above-mentioned two types, such that more accurate results can be obtained by developing more comprehensive and efficient CTC detection approaches.

2. Immunomagnetics

Almost all cells are either diamagnetic or very weakly magnetic. Hence, a magnetic field can be used to effectively isolate CTCs from the blood if their magnetic property is selectively modified. Magnetic CTC enrichment methods are low-cost, easy-to-operate, high-sensitivity, and thus suitable to operate in any biological environment. CTCs can be tagged using antibody-conjugated magnetic microbeads or nanoparticles that often bind to a specific surface antigen and using permanent magnets to drive the labeled CTCs for separation. An overexpressed

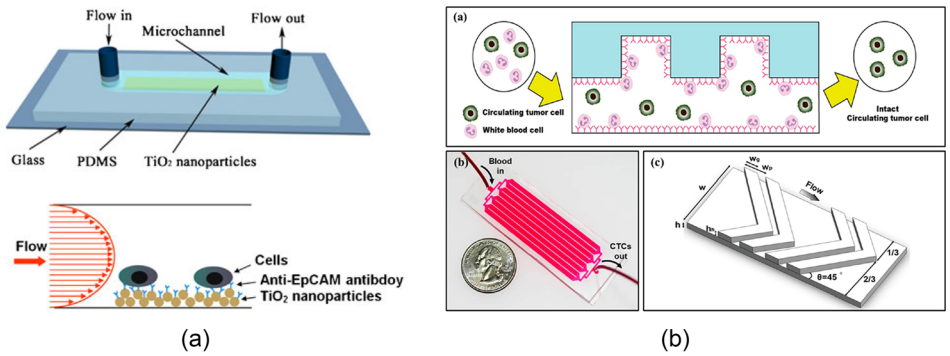


FIG. 8. (a) A biocompatible and surface roughness controllable nano-film for highly efficient CTC capture.[102] Reproduced with permission from He *et al.*, Biomed. Microdevices **15**, 617 (2013). Copyright 2013 Springer. (b) A geometrically activated surface interaction chip using a herringbone shape to efficiently capture a large number of hematological cells rather than CTCs.[103] Reproduced with permission from Hyun *et al.*, Anal. Chem. **85**, 4439 (2013). Copyright 2013 American Chemical Society.

surface antigen could increase the uptake of immunomagnetic particles, which reinforces the magnetic force acting on cells and facilitates their isolation. EpCAM has often been used for this purpose. Another approach, which is independent of the phenotype of CTCs, is to negatively isolate CTCs by lysing red blood cells (RBCs) and using specific markers (e.g., CD45 or CD61) to magnetically remove white blood cells (WBCs) from the sample. Immunomagnetic assay has shown clinical significance in cancer diagnosis and prognosis.[104–109] The generation and fine-tuning of the magnetic field play essential roles in such assay toward effective single-cell-based analyses.

However, the current assay has a limited range of field gradients, potentially leading to an aggregation of the cells and nanoparticles. In order to amplify the magnetic field gradient, Huang *et al*. developed a micromagnet-integrated microfluidic system for enhanced CTC detection (Fig. 9).[110] The ferromagnetic micromagnets, after being magnetized, generate localized magnetic field up to 8-fold stronger than that without the micromagnets and strengthen the interactions between CTCs and the magnetic field. The system was demonstrated with four cancer cell lines to achieve over 97% capture rate. The system captures target CTCs from a patient's blood samples on a standard glass slide that can be examined using the fluorescence *in-situ* hybridization method for the single-cell profiling.

The efficiency of immunomagnetic CTC enrichment is determined by three factors, including (a) the expression and specificity of the target antigen and the binding quality of the associated antibody, (b) the efficiency of immunomagnetic labeling process and magnetic particles, and (c) the separation mechanism designed to isolate labeled cells. To further improve efficiency, significant efforts are made in the research of these three aspects.

III. SINGLE CELL CAPTURE

Single cell handling is essential for research and application development due to noticeable variations across individual cells, such as the genetic expressions. To study these variations, therefore, after the separation of CTCs from other populations of cells, it is normally required to capture single CTCs from the CTC medium for further analysis. A variety of techniques have been employed to trap an individual cell within a microfluidic device in response to an increasing demand for high-throughput cell manipulation at the single cell level, including mechanical/hydrodynamic traps, magnetic traps, optical traps, DEP traps, acoustic traps and droplets.

A. Mechanical/hydrodynamic traps

Hydrodynamic traps utilize specific microstructures and valves in a microfluidic channel to control the fluid flow so as to collect single cells without the use of other apparatus. This method is simple, but the fabrication of a microfluidic channel might be complicated because of microstructures. Specially designed mechanical micropillars placed in microchannels can trap single cells in array.[111,112] Zhang *et al*. introduced a live single cell printing method (Fig. 10(a)).[113] The approach allows for convenient and highly efficient formation of multiplexed

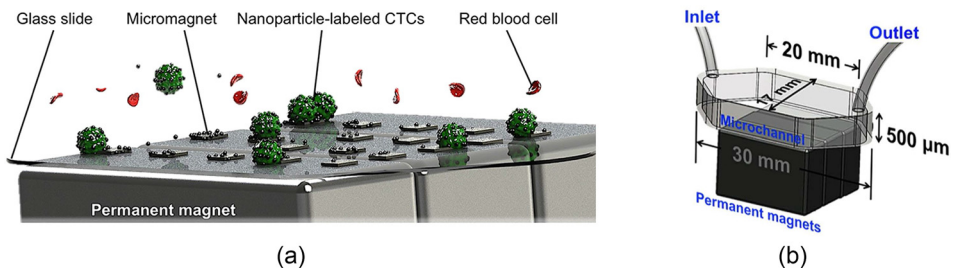


FIG. 9. Micromagnet-integrated microfluidic device and characterization of micromagnets. (a) Schematic illustration of the glass substrate patterned with micromagnets for immunomagnetic isolation of cancer cells. (b) Schematic shows the setup of the screening system with dimensions of the microchannel.[110] Reproduced with permission from Huang *et al*., Sci. Rep. **5**, 16047 (2015). Copyright 2015 Nature Publishing Group.

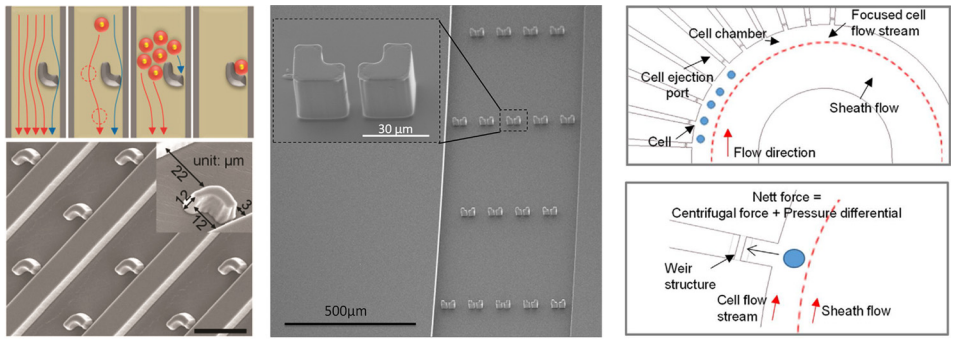


FIG. 10. (a) A block single-cell printing method for a convenient and highly efficient formation of multiplexed single-cell arrays.[113] Reproduced with permission from Zhang *et al*., Proc. Natl. Acad. Sci. U. S. A. **111**, 2948 (2014). Copyright 2014 National Academy of Sciences. (b) A microfluidic device for single cell analysis with U-shape mechanical traps.[114] Reproduced with permission from Benavente-Babace *et al*., Biosen. Bioelectron. **61**, 298 (2014). Copyright 2014 Elsevier. (c) A microfluidic device is capable of high-throughput specific selection and isolation of single rare cells. A single cell could be focused, captured and recovered by utilizing hydrodynamic and positive pressure.[116] Reproduced with permission from Yeo *et al*., Sci. Rep. **6**, 22076 (2016). Copyright 2016 Nature Publishing Group.

single-cell arrays with precise, adjustable cell spacing, sophisticated single-cell patterning, coculture of heterotypic cell pairs, and an elongated cell array. Benavente-Babace *et al*. created uniform arrays of trapped single cells in a microfluidic device (Fig. 10(b)).[114] The surrounding flows of the U-shaped mechanical traps would be changed before and after cell loading, which resulted in the temporally efficient cell loading of empty traps.

Trapping single cells in microfluidic systems often relies on the design of side hooks to a main transport channel, where the side hooks are small enough to trap cells by differential pressure.[115] Yeo *et al*. developed a microfluidic device that is able to perform high-throughput, specific, picking and enrichment of single CTCs (Fig. 10(c)).[116] This device based on hydrodynamic focusing can direct single cells into single cell chambers with the help of a viscous sheath flow buffer. The cells are ushered into the holding chambers due to the inherent differential pressure at these chambers. Because cell chambers are lined along the outer curvature of the channel, cells will experience slight centrifugal force that facilitates their entry into these chambers. For cell recovery, a positive pressure is exerted through a particular chamber to push the selected cell back into the main stream and into a collection port. By combining centrifugal capture and positive pressure, the device can quickly and efficiently trap single cells and has the flexibility to transfer any cell or cells of interest for downstream analysis. This keeps the closeness of the channel for easy operation in microfluidics, while needing an optimization of the design for housing more chambers to increase throughput with the reduced number of valves.

Hydrodynamic trapping also uses the altered fluidic resistance created by microstructures on a fluid path, such as small trapping sites to control the movement of cells in a microchannel. Jin *et al*. designed a ladder-like microchannel that can high-efficiently capture single cells, which is based on the least flow resistance principle (Fig. 11(a)).[117] The flow resistances of the channels are carefully calculated, so that the fluid and cells in the main channel will be directed into the trapping sites when the channels are empty but bypass the trap sites when a single cell is captured. The main challenge in hydrodynamic trapping is that it requires a precise microfluidic control of multiple streams and further investigation and optimization of cell trapping efficiencies are still required. Although the simple device works well, it suffers from cell blockage and low-efficiency. Recently, Mi *et al*. presented a novel single cell trap design and device with a matrix of cell trap units inspired on an equivalent resistive electric circuit (Fig. 11(b)).[118] The device can achieve deterministic cell trapping, which could serve as a powerful enabling tool for single cell analysis especially when the quantity of sample cells is quite low, possessing a highly flexible structure compatible with existing 96- (or 384-) or even higher density well plates, and demonstrating easy-to-implement capability of cell patterning at a large scale of 10^4. As each individual trap unit works independently and equivalently, the topology of the device can be adjusted at

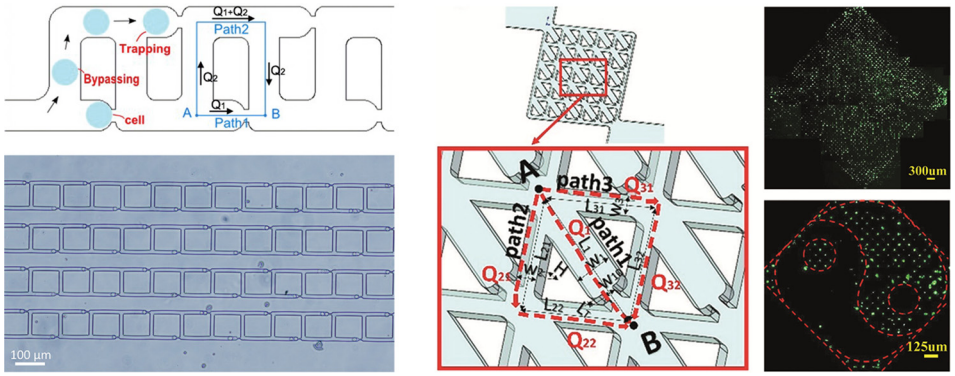


FIG. 11. Hydrodynamic single cell trapping using fluidic resistance principle. (a) Design and experiment of ladder-like microfluidic devices for single cell trapping.[117] Reproduced with permission from Jin *et al.*, Biomicrofluidics **9**, 14101 (2015). Copyright 2015 AIP Publishing. (b) Working principle and experimental results of the fluidic circuit based microfluidic device for single cell trap.[118] Reproduced with permission from Mi *et al.*, Lab Chip **16**, 4507 (2016). Copyright 2016 Royal Society of Chemistry.

will and the number of trap units can be scaled up on demand as well. However, one great challenge is how to selectively address and manipulate (e.g., transfer) the cell on one particular unit for downstream analysis. To tackle this challenge, the integration of more microfluidic modules on one chip is a possible solution like the work in the previous paragraph.

B. Magnetic traps

As mentioned previously in the cell enrichment, target cells could be tagged using the antibody-conjugated magnetic microbeads or nanoparticles. Once the magnetically labeled target cells are exposed to a non-uniform magnetic field, the field gradient forces them to migrate towards the regions with the highest magnetic flux density.[119–121] Both the labeling protocol (i.e., direct or indirect labeling) and the magnetic particles (i.e., size and composition) can affect the performance of immunomagnetic methods.[122] The magnetic force exerted on magnetically labeled cells partly depends on the strength and gradient of the flux density produced by the magnetic source, which could be an electromagnet, a permanent magnet, or some soft magnetic material that should in turn be magnetized by a primary source. Magnetic flux density peaks can be generated by designing different geometry of micro patterns and used to capture the cells at the area of peak spots. The magnetic flux density drops quickly ($\propto 1/r^3$) as the distance (r) from the source increases, reducing the force experienced by labeled cells. Thus, the key to trapping a single magnetic cell[123] is to generate the peaks at a spatial resolution comparable to the cell dimension and pattern the on-chip magnetic sources to produce desired magnetic flux. Esmaeilsabzali *et al.* proposed an integrated microfluidic chip containing the multiple arrays of permalloy-based magnetic microtraps for immunomagnetic isolation of prostate cancer cells (Fig. 12(a)).[124] The chip consists of a single layer of soft magnetic material with high magnetic permeability that has been electroplated onto a substrate. The single layer is configured with thousands of sawtooth-shaped magnetic microtraps. Therefore, in the presence of an external magnetic field, a peak of magnetic flux density is generated in the valley between the teeth, where a magnetically labeled cell could be trapped. The device demonstrated a capture rate from 83% to 98% for different cell density (20–200 cells/ml).

Magnetic traps can be extended with a carefully designed local magnetic field to manipulate cells at sub-micron resolution. Recently, the use of domain walls (DWs) in magnetic conduits patterned on a chip has been proven viable to achieve highly controllable motion of single micro and nanoparticles.[125] DWs in magnetic nanostructures provide localized sources of a strong magnetic field gradient used to trap and externally manipulate individual cells.[126] Vieira *et al.* utilized adjacent ferromagnetic zig-zag wires, where static DWs were created at each

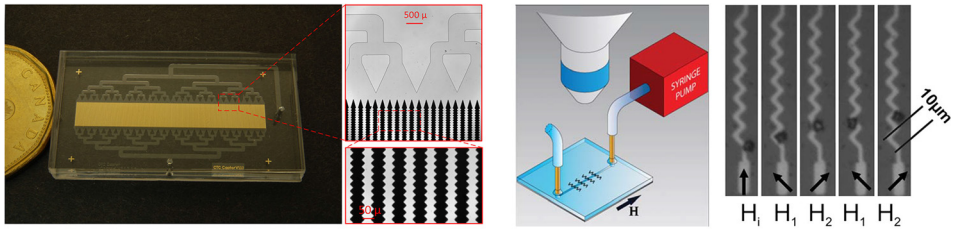


FIG. 12. Single cell trapping via magnetic methods. (a) An integrated microfluidic chip containing multiple arrays of permalloy-based magnetic microtraps for immunomagnetic isolation of prostate cancer cells.[124] Reproduced with permission from Esmaeilsabzali *et al.*, Biomed. Microdevices **18**, 22 (2016). Copyright 2016 Springer. (b) A platform for trapping and manipulating single cells based on magnetic domain wall tweezers.[128] Reproduced with permission from Donolato *et al.*, Lab Chip **11**, 2976 (2011). Copyright 2011 Royal Society of Chemistry.

corner to generate a bidimensional array of traps to immobilize T-lymphocyte cells.[127] Furthermore, Donolato *et al.* achieved a controllable transport and release method of individual yeast cells via the remote manipulation of individual domain walls in micro- and nano-sized magnetic conduits (Fig. 12(b)).[128] The precision of cell transport is down to the 100 nm scale using nano-sized magnetic conduits. Long-range transport of cells can reach over 100 μm using micro-sized conduits.

Magnetic traps for single cell trap are useful since a static magnetic field does not disturb the movement of ions in the culture medium. Therefore, it is suitable to use in conjunction with other electrical cell analysis techniques. Unlike DEP and some hydrodynamic methods, the magnetic field does not induce trans-membrane voltage, Joule heating or shear stress on the cell. Thus, it is amenable for the prolonged immobilization of cells and ideal for long-term cell experiments such as cell function assays and rare event detection.

C. Optical traps

Due to non-contact and contamination-free manipulation process, optical tweezers can be easily combined with microfluidic systems to trap single cells.[129–133] Optical tweezers manipulate cells with very high precision and possibility to spatially arrange cells. Microscale objects are forced by optical forces towards the focus point of a laser beam, where the trapped objects can be repositioned in all dimensions by moving the beam and changing focus.[134] Prolonged handling times in small volumes can pose a problem due to the fact that the absorbed laser energy in the water-based buffers may lead to harmful Joule heating of the cells. Liberale *et al.* proposed a novel and fully integrated system with the miniaturized fiber-based optical tweezers to achieve stable 3D-trapping through the micro-prism beam deflectors fabricated by two-photon lithography (Fig. 13(a)).[135]

The new generation of optical tweezers outperforms the normal single beam-single trap system in an enhanced throughput, i.e., operating multiple objects. A limitation in the normal optical tweezers' setup was the fact that a separate laser beam was required for every object to manipulate. The manipulation of multiple objects thus requires complicated and expensive setup, which limited applications of this technique. In an attempt to improve the throughput, Yevnin *et al.* proposed a dual-objective approach to combine 3D holographic optical tweezers with a spinning-disk confocal microscope,[136] but the setup is rather expensive. To provide an economic solution, Werner *et al.* reported on the development of a microfluidic array cytometer based on refractive multiple optical tweezers generated by the inexpensive microlenses (Fig. 13(b)).[137] They demonstrated the ability of such optical trap arrays to immobilize more than 200 yeast cells in parallel. Single cells immobilized in the array could be individually manipulated and isolated. This new capability was enabled by the use of microlens, which has a high damage threshold offering the possibility to use the high-power lasers in the system. Low cost and easy integration of the microlens into the microscopy imaging system would promote its application and further improve the throughput and controllability.

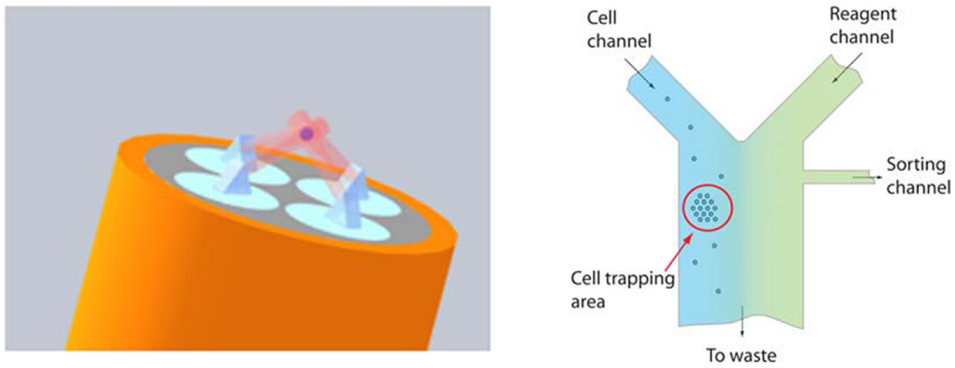


FIG. 13. (a) A fully integrated system relying on miniaturized fiber-based optical tweezers that achieves stable 3D-trapping.[135] Reproduced with permission from Liberale *et al*., Sci. Rep. **3**, 1258 (2013). Copyright 2013 Nature Publishing Group. (b) A sorting tool based on refractive multiple optical tweezers combined with microfluidics and optical microscopy.[137] Reproduced with permission from Werner *et al*., Lab Chip **11**, 2432 (2011). Copyright 2011 Royal Society of Chemistry.

D. Dielectrophoretic traps

Dielectrophoresis-based cell trapping relies on a non-uniform AC field to manipulate single cells in suspension and is effective for manipulating a single cell. The strength of DEP forces is dependent on the shape of electrodes, so an electrode tip with greater DEP force can capture, convey, and position single cell, as shown Fig. 14(a).[138] The cell could be captured by the positive DEP force and released by the negative DEP force.[139,140] Similarly, the electric field cages or wells can be formed by special electrode shape and signal configuration to capture single cells.[141–143] However, this method needs visual feedback and complex control and would totally stop working once out of the liquid environment.

The precise translation and rotation of biological entities are two fundamental manipulation requirements in the applied biotechnological research. However, rotation remains extremely challenging when there is a need for multiple-axis rotation to adjust the cell orientation in 3D space. Benhal *et al*. presented a novel structure that can achieve 3-D rotation in one single chip (Fig. 14(b)).[71] A single cell located within an electrode chamber can have in-plane or rolling rotation in association with different ac signal configurations. However, loading a single cell into electrode chamber is a difficult problem, which limited the experiment efficiency. In order to overcome the loading problem, Huang *et al*. presented a 3D rotation platform that integrated DEP and microfluidic technology (Fig. 14(c)).[72] Based on the least flow resistance principle, one single cell can be captured at trap site and translated into the electrode chamber for 3D rotation. Furthermore, using unique signal configuration can facilitate in-plane cell centering in the rotation chamber and prevent the rotating cell from sinking down to the bottom.

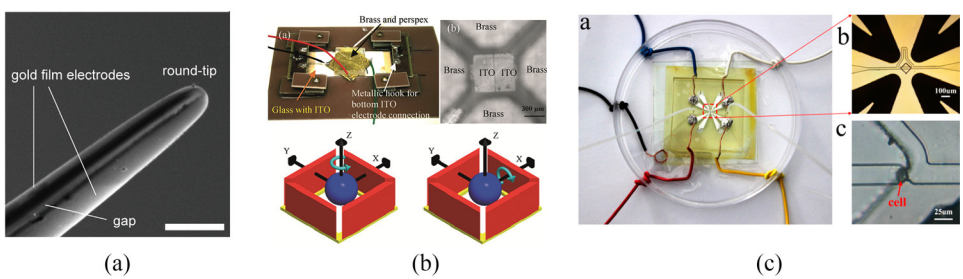


FIG. 14. Electrode structures with DEP force for manipulating single cell. (a) A round-tip DEP-based tweezers for trapping single cell.[138] Reproduced with permission from Kodama *et al*., Biosen. Bioelectron. **47**, 206 (2013). Copyright 2013 Elsevier. (b) A 3D rotation biochip platform.[71] Reproduced with permission from Benhal *et al*., Lab Chip **14**, 2717 (2014). Copyright 2014 Royal Society of Chemistry. (c) A 3D rotation platform that integrated DEP and microfluidic technology.[72]

Microwell arrays coupled with microelectrodes can further improve the throughput and efficiency in single cell capture. The DEP force in the selected dimensions of the microwells could yield efficient trapping in nearly all the microwells.[144] Once single cells were trapped in the microwells by DEP force, DEP signals could be switched off, whereas the microwells hold cells on-site even when unstable flow of liquid is applied. In this way, it is possible to substitute the DEP buffer with a solution that will be subsequently used for stimulating or analyzing the trapped cells. Kim *et al.* presented a microfluidic device containing a large array of electroactive microwells that performs DEP-based single cell trapping and subsequent cell lysis (Fig. 15(a)).[145] This demonstrated that DEP force not only provides fast, active and highly efficient trapping but also holds cells *in situ* against unstable flow. Recently, this concept was extended to the larger-scale manipulation of cell pairing and electroporation-based fusion. Mustafa *et al.* presented a device with an array of 900 gourd-shaped microwells designed to pair single cells of different types (Fig. 15(b)).[146] Each side of a microwell stays on a different comb of the interdigitated array, so that the cells of different types are trapped on opposite sides of the microwells, forming cell pairs. Using this device, a large number of cell pairs can be formed easily and rapidly, making it a highly attractive tool for controllable cell pairing in a range of biological applications.

E. Acoustic traps

Surface acoustic wave (SAW)-based methods are often used for cell sorting and can also be used for non-contact trapping of single microparticles, cells, and entire organisms.[147–149] The acoustic technique has three merits: (a) the capability of manipulating most microparticles, regardless of optical, electrical, magnetic, or shape properties; (b) the ability to manipulate objects with a range of scales, from nanometer to millimeter; and (c) the capability of rapidly manipulating a single particle or groups of particles.

The SAW methods that integrate with advanced fluid control make it possible to handle single cells in a non-contact mode. Cells in a standing acoustic field experience an acoustic

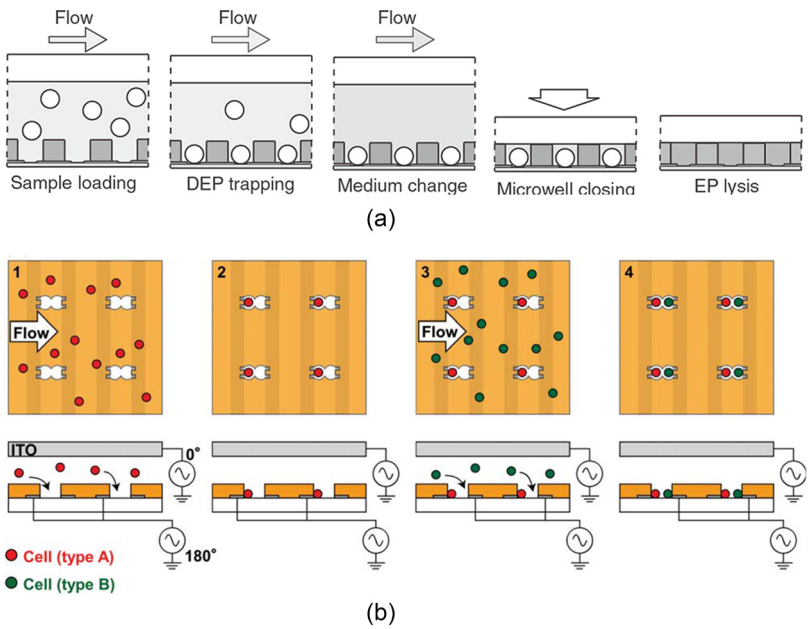


FIG. 15. (a) A microfluidic device containing a large array of electroactive microwells that performs DEP-based single cell trapping and subsequent cell lysis.[145] Reproduced with permission from Kim *et al.*, Small 7, 3239 (2011). Copyright 2011 John Wiley and Sons. (b) A chip device with an array of gourd-shaped microwells designed to pair single cells.[146] Reproduced with permission from Sen *et al.*, Lab Chip **13**, 3650 (2013). Copyright 2013 Royal Society of Chemistry.

radiation force that pushes them toward the pressure nodes. Using this mechanism, one can generate a standing SAW field using the pairs of aligned IDTs to pattern cells in one or two dimensions. The trapping nodes could be precisely manipulated by adjusting the phase angle and the excitation frequencies of each individual IDT pair. When the acoustic wavelength is on the same order as the cell dimensions, single cells will be patterned in one or two dimensions.[150] However, using 2D acoustic waves often results in insufficient control of a single cell in 3D space. Due to the limited understanding of the relationship between a 3D acoustic field and the induced acoustic streaming, there is no acoustic approach that has hitherto demonstrated the controlled 3D manipulation of single cells. Recently, Guo *et al.* demonstrated the controlled 3D manipulation of single cells by using the pairs of aligned IDTs (Fig. 16(a)).[151] They used SAW to pick up single cells, or cell aggregates, and deliver them to desired locations to create 2D and 3D cell patterns or print the cells into complex shapes in 3D. The coordinates of 3D trapping nodes were precisely controlled by adjusting the phase angle of each individual IDT pair (for x or y coordinate) or the input acoustic power (for z coordinate). Amazingly, the positioning accuracy of single cells was down to 1-μm in the x-y plane and 2-μm in the z direction. Although SAW for single cell trap is powerful, acoustic manipulation such as picking an individual cell from the trapped cells and transferring it to the downstream analysis step has been difficulty, as the acoustic nodes are hardly controlled separately. There is much room here for improvement.

In addition for cell patterning, acoustic traps can also be extended to measure the mechanical properties of cells. The potential of single beam acoustic tweezers (SBAT) has been shown in various biomedical applications as an acoustic manipulation platform, such as cell deformation and stimulation. Compared to the optical tweezers, the SBAT technique offers several advantages, such as generating stronger force at nano-Newton level and causing less cell damage. It has been demonstrated that SBAT could trap the objects with a size either larger or smaller than an acoustic wavelength.[152,153] Hwang *et al.* proposed a non-contact single-beam acoustic trapping method for the quantification of the mechanical properties of a suspended cell without any materials attached to the cell (Fig. 16(b)).[154] The isolation of a suspended target cell from other adjacent cells is achieved by the acoustic trapping of the target cell using a 30 MHz lithium niobate ($LiNbO_3$) highly focused ultrasound transducer. The deformation of a trapped cell is related to the applied acoustic pressure and cell mechanical properties. SBATs could also be employed to study the properties of cancer cell membrane deformation.[155] However, there are still some shortages of SBAT need to be overcome: more efforts should be made to quantify the force generated from the SBATs; the three dimensional manipulation is desired and challenging.

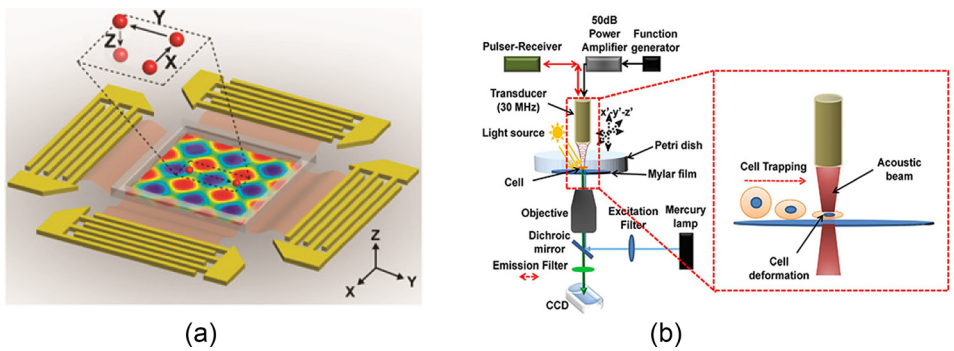


FIG. 16. (a) A SAW microfluidic device that creates 3D trapping nodes for the capture and manipulation of microparticles and cells along three mutually orthogonal axes.[151] Reproduced with permission from Guo *et al.*, Proc. Natl. Acad. Sci. U. S. A. **113**, 1522 (2016). Copyright 2016 National Academy of Sciences. (b) An SBAT method for the measurement of the mechanical properties of a suspended cell.[154] Reproduced with permission from Hwang *et al.*, Sci. Rep. **6**, 27238 (2016). Copyright 2016 Nature Publishing Group.

F. Droplets

Droplet-based microfluidic systems for single-cell analysis are adopted for many different applications, mainly due to two attractive advantages: the dimensional scaling benefits and the benefits of enclosed individual microenvironment. Micro droplet chambers, with the volume ranging from femtoliter to nanoliter, have the merits of low cost and high throughput (the generation rates of droplets vary from 100 Hz to 100 kHz). More importantly, on the one hand, heat and mass transfer as well as diffusion would be enhanced with higher surface area to volume ratio at microscale, and thus benefit biochemical process of individual cells inside droplets. On the other hand, the confinement of cells inside droplets as well as the immiscible interface between two phases provides chemical isolation of each cell, which is necessary for long-term analysis since, in the enclosed microenvironment, some undetectable signals of cells could be concentrated to the measurable level without contamination.[156,157] The most commonly used aqueous monodispersed droplets are usually formed in continuous pressure-driven nozzles with flow focusing[158] (Fig. 17(a)), co-flowing[159] (Fig. 17(b)) or T-junction[160] designs (Fig. 17(c)), and in addition, droplets could also be formed by electrical control, such as electrowetting on dielectric (EWOD).[161]

The main challenges in droplet-based single cell analysis are to maintain the integrity of the chambers and the viability of encapsulated cells. For example, undesired coalescence and break-up of droplets, nutrient depletion and the accumulation of toxic metabolites need to be overcome for long-term analysis. To solve these problems, many fundamental studies have been conducted. Some of them focus on flow physics of droplets, such as droplet break-up in a concentrated emulsion flowing through a narrow constriction.[162] Some researchers are interested in the interface between the droplets and the continuous phase. For example, Pan *et al*. replaced the traditional surfactant with nanoparticles as droplet stabilizer, which would mitigate the undesired cross-contaminations of droplet content caused by the inter-drop transport of small hydrophobic molecules.[163] Fundamental research is still necessary since it would deepen our understanding about how to design a particular microenvironment for single cell analysis. In parallel, many researchers focus on the high-throughput applications of droplet-based microfluidic systems. In a recent effort, Klein *et al*. reported a high-throughput droplet-microfluidic approach for barcoding the RNA from thousands of individual cells for subsequent analysis by the next-generation sequencing (Fig. 17(d)).[164] The method shows a surprisingly low noise profile and is readily adaptable to other sequencing-based assays. Such a method is suitable for

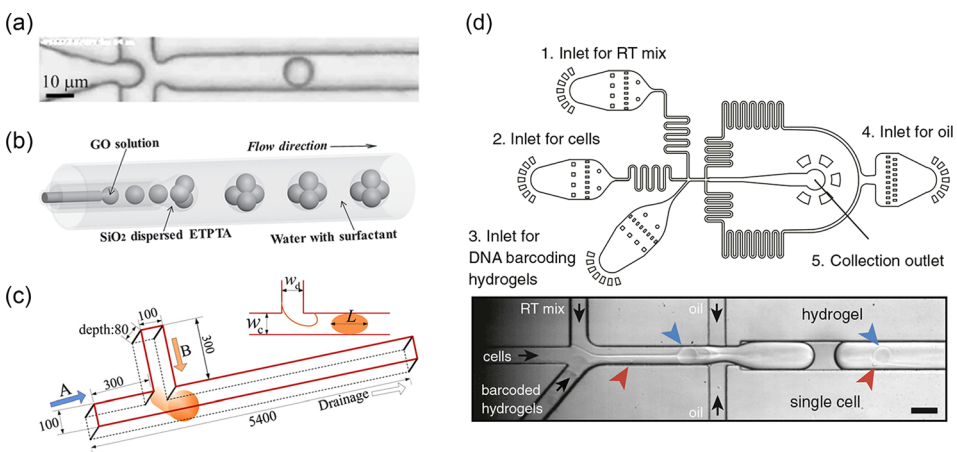


FIG. 17. Droplets formed in continuous pressure-driven nozzles by (a) flow focusing,[158] reproduced with permission from Weinmeister *et al*., ACS Nano **9**, 9718 (2015). Copyright 2015 American Chemical Society. (b) Co-flowing,[159] reproduced with permission from Wang *et al*., Small **11**, 3890 (2015). Copyright 2015 John Wiley and Sons. (c) T-junction,[160] reproduced with permission from Li *et al*., Chem. Eng. Sci. **69**, 340 (2012). Copyright 2012 Elsevier. (d) A high-throughput droplet-microfluidic approach for barcoding the RNA from thousands of individual cells.[164] Reproduced with permission from Klein *et al*., Cell **161**, 1187 (2015). Copyright 2015 Elsevier.

small clinical samples including from tumors and tissue microbiopsies and opens up the possibility of routinely identifying cell types, even if rare, based on gene expression.

However, these high-throughput applications still face some challenges basically from two aspects: sample preparation and content analysis of droplets. Although the state-of-art technologies make droplet generation easy, fast and cheap, to avoid multiple-cell-in-one-droplet, cells usually occupy only 10% or less of droplets, which sacrifices the overall efficiency. So, how to insert a single cell into a droplet with high efficiency is still a problem that should be solved. To solve this problem, probably more fundamental research in physics of microfluidics is required. On the other hand, because droplets are small enough, usually biochemical reading of super-tiny droplet content needs complex optical systems with large lens, which means miniaturization is also a challenge. Some lens-free technique may solve this problem, such as optofluidic microscopy (OFM). Droplet microfluidics offers significant advantages for high-throughput screenings and sensitive assays, allowing sample volumes to be significantly reduced. It has a powerful potential to support scientific progress in further analysis of the cells. Interested readers can refer to the themed reviews[165,166] for more information.

IV. PERSPECTIVE

Single cell analysis calls for ingenious solutions to cell sample preparation. For such two key preparation tasks of high-efficiency cell enrichment and precise single cell capture, a rich library of technologies is available as reviewed above and innovative advances continue to emerge, providing a wealth of research and application opportunity to both scholars and practitioners in this field. Most of the above-mentioned technologies can be used both for the two tasks, after proper and easy alteration in configurations. Below, a brief appraisal of each technology is given as a perspective.

The filtration-based technology is simple and specifically effective for separating size-differentiable cells. It is rather straightforward for a cell enrichment platform to be built up, and the throughput has been improved significantly with some serious issues like cell clogging or air bubbles already eliminated or alleviated. However, the challenges remain in making a compromise among important performance indicators such as enrichment ratio, purity, throughput, efficacy, cell viability, clogging, and air bubbles. Similarly, the hydrodynamic enrichment technology also relies on the size variations of cell types. But its simper microfluidic structures exclude the need of porous membranes, which require rather expensive fabrication process. Due to the easy scaling-up, its throughput has recently reached record-breaking high-level for clinical trials. From this aspect, hydrodynamic technology holds advantageous potential and has found some commercialization trials at the clinic in CTC enrichment. In single cell trap, the hydrodynamic technology relies on some specifically designed structure to capture one single cell out of the cell medium flowing through a channel with a probability. It evolves along two directions: (1) random capture of cells for ample cells and (2) deterministic capture of cells for rare cells, both toward large-scale and flexible patterning of cells and integration with single cell transfer module to facilitate subsequent cell analysis.

DEP technology separates and captures cells by their different electrical properties, featuring high specificity and cell viability. At the earlier stage, the fabrication of DEP electrodes was challenging and now advances in MEMS fabrication technologies facilitate flexible and large-arrayed configurations of electrodes together with microfluidic channels. Both traditional DEP and iDEP technologies can work to produce DEP effect for cell enrichment. In this regard, opportunities lie in optimized design of electrodes and insulating microfluidic structures such that a low (e.g., <10 V) DEP voltage is sufficient. The benefits of using a low potential are to simplify the peripheral electronics, reduce the Joule-heating effect and air-bubble generation.

Optical tweezers are very competent for precisely manipulating single cells, including single cell trap. In the course of cell enrichment, optical tweezers can make use of the refractive index, size or shape of the cells as the indicators in separation. For cells that do not exhibit particularly different refractive index like CTCs, optical tweezers work in an active mode such that CTCs have to be fluorescence tagged and feedback control is required by using a sensor to

screen target cells. The involvement of an external sensor complicates the system, and the one-beam-one-trap nature of optical tweezers makes it a low throughput tool for cell enrichment, but a precise one for single cell trap.

Acoustic technology separates and captures cells using the acoustic radiation pressure at low cost, high controllability and biocompatibility. Like other physical technologies, it does not label cells. Furthermore, by tuning the frequency of standing acoustic waves, the wavelength can become comparable to cell dimension such that one single cell can be immobilized on the node. The trend for acoustic technology is to design and fabricate highly focused SAW for cell enrichment purpose and to use high-frequency acoustics to increase the spatial resolution for trapping particles, including single cells. Capable of penetrating deep tissue, acoustic technology has the potential for *in vivo* applications. But the selectivity of cell size for acoustic enrichment is poor. Probably, the selectivity can be improved by tagging cells with some selective markers, like for active-mode optical tweezers.

Magnetic technology can be used for both cell enrichment and single cell trap, by tagging cells with magnetic beads as an invasive method. Advances in antibody-antigen specificity and expression and efficiency of the magnetic labeling processes are highly expected to make the tagging selective and effective. In both cell enrichment and single cell trap, opportunities lie in the design of device structure that can produce locally sensitive, controllable, and sufficiently strong magnetic fields to separate a cell population or a single cell. The fabrication of magnetic materials to micropatterns on chip is challenging as well. Like magnetic technology, affinity reaction technology is also based on the specificity and expression of the antigen-antibody locking mechanism to enrich a specific type of cells. Therefore, the associated challenges are largely similar to that of magnetic technology in biomarker identification, expression level, and antigen-antibody affinity.

Droplets are very powerful in single cell trap and highly competitive for subsequent single cell analysis, such as proteomics, metabonomics, and sequencing. Fundamental problems still exist in droplet mechanisms, such as how to make multi-phase droplets and maintain their long-term stability. However, these do not hamper droplets from being applied for many exciting single cell analysis applications, where the opportunity is ample for analytical instrument developers and practitioners.

In summary, the technologies in this review each have their own specific challenges, which can be regarded as research opportunities in their own area. However, an increasing need arises for integrating multiple of them to perform complex manipulation and analytical tasks on one platform. The need comes from bedside diagnosis, personalized healthcare and point-of-care tests (POCTs). Hence, sample rare cells, like CTCs, have to be enriched and manipulated accurately in preparation for downstream analysis. The preparation process should also be of low cost and high efficiency that will be suitable for possible commercialization. According to the advantages of microfluidics, several technologies can be used on a chip simultaneously to achieve multiple functions and high performance (such as high purity and high accuracy). Therefore, many efforts have to be made for each technology to be easily integrated. By doing so, integrated cell manipulation and analysis platforms will conveniently be assembled and readily accepted by end-users, such as doctors, biologists, and analysts, playing a greater role in cancer and non-cancer cell studies.

ACKNOWLEDGMENTS

This work was supported by the NSFC (No. 61376120), the National Key R&D Program (No. 2016YFC0900200), the National Instrumentation Project (No. 2013YQ19046701), and the One-Thousand Young Talent Program of China.

[1]M. Spitzer and G. Nolan, Cell **165**, 780 (2016).
[2]P. Haverty, E. Lin, J. Tan, Y. Yu, B. Lam, S. Lianoglou, R. Neve, S. Martin, J. Settleman, R. Yauch, and R. Bourgon, Nature **533**, 333 (2016).
[3]A. Scialdone, Y. Tanaka, W. Jawaid, V. Moignard, N. Wilson, I. Macaulay, J. Marioni, and B. Göttgens, Nature **535**, 289 (2016).

[4]O. Guillaume-Gentil, R. Grindberg, R. Kooger, L. Dorwling-Carter, V. Martinez, D. Ossola, M. Pilhofer, T. Zambelli, and J. Vorholt, Cell **166**, 506 (2016).
[5]J. Mahamid, S. Pfeffer, M. Schaffer, E. Villa, R. Danev, L. Cuellar, F. Förster, A. Hyman, J. Plitzko, and W. Baumeister, Science **351**, 969 (2016).
[6]T. Gorges, A. Kuske, K. Rock, O. Mauermann, V. Muller, S. Peine, K. Verpoort, V. Novosadova, M. Kubista, S. Riethdorf, and K. Pantel, Clin. Chem. **62**, 1504 (2016).
[7]K. Mann, J. Newberg, M. Black, D. Jones, F. Amaya-Manzanares, L. Guzman-Rojas, T. Kodama, J. Ward, A. Rust, L. van der Weyden, C. Yew, J. Waters, M. Leung, K. Rogers, S. Rogers, L. McNoe, L. Selvanesan, N. Navin, N. Jenkins, N. Copeland, and M. Mann, Nat. Biotechnol. **34**, 962 (2016).
[8]K. Frieda, J. Linton, S. Hormoz, J. Choi, K. Chow, Z. Singer, M. Budde, M. Elowitz, and L. Cai, Nature **541**, 107 (2016).
[9]S. Krishnaswamy, M. Spitzer, M. Mingueneau, S. Bendall, O. Litvin, E. Stone, D. Pe'er, and G. Nolan, Science **346**, 1079 (2014).
[10]D. Lawson, N. Bhakta, K. Kessenbrock, K. Prummel, Y. Yu, K. Takai, A. Zhou, H. Eyob, S. Balakrishnan, C. Wang, P. Yaswen, A. Goga, and Z. Werb, Nature **526**, 131 (2015).
[11]S. Tay, Cell **162**, 1208 (2015).
[12]H. J. Yoon, M. Kozminsky, and S. Nagrath, ACS Nano **8**, 1995 (2014).
[13]C. Zong, S. Lu, A. Chapman, and X. Xie, Science **338**, 1622 (2012).
[14]J. Buenrostro, B. Wu, U. Litzenburger, D. Ruff, M. Gonzales, M. Snyder, H. Chang, and W. Greenleaf, Nature **523**, 486 (2015).
[15]L. Mazutis, J. Gilbert, W. Ung, D. Weitz, A. Griffiths, and J. Heyman, Nat. Protoc. **8**, 870 (2013).
[16]M. Warkiani, B. Khoo, L. Wu, A. Tay, A. Bhagat, J. Han, and C. Lim, Nat. Protoc. **11**, 134 (2016).
[17]X. Fan, C. Jia, J. Yang, G. Li, H. Mao, Q. Jin, and J. Zhao, Biosen. Bioelectron. **71**, 380 (2015).
[18]W. Duncanson, T. Lin, A. Abate, S. Seiffert, and R. Shah, Lab Chip **12**, 2135 (2012).
[19]T. Konry, M. Dominguezvillar, C. Baecherallan, D. Hafler, and M. Yarmush, Biosen. Bioelectron. **26**, 2707 (2011).
[20]G. Vladisavljević, I. Kobayashi, and M. Nakajima, Microfluid. Nanofluid. **13**, 151 (2012).
[21]F. Difato, G. Pinato, and D. Cojoc, Int. J. Mol. Sci. **14**, 8963 (2013).
[22]M. Habaza, B. Gilboa, Y. Roichman, and N. Shaked, Opt. Lett. **40**, 1881 (2015).
[23]S. Schuerle, S. Erni, M. Flink, B. E. Kratochvil, and B. J. Nelson, IEEE Trans. Magn. **49**, 321 (2013).
[24]L. Chang, M. Howdyshell, W. Liao, C. Chiang, D. Gallego-Perez, Z. Yang, W. Lu, J. Byrd, N. Muthusamy, L. Lee, and R. Sooryakumar, Small **11**, 1818 (2015).
[25]A. Salmanzadeh, L. Romero, H. Shafiee, R. Gallo-Villanueva, M. Stremler, S. Cramer, and R. Davalos, Lab Chip **12**, 182 (2012).
[26]S. Shim, K. Stemke-Hale, A. Tsimberidou, J. Noshari, T. Anderson, and P. Gascoyne, Biomicrofluidics **7**, 11807 (2013).
[27]Y. Qiu, H. Wang, C. Demore, D. Hughes, P. Glynne-Jones, S. Gebhardt, A. Bolhovitins, R. Poltarjonoks, K. Weijer, A. Schönecker, M. Hill, and S. Cochran, Sensors **14**, 14806 (2014).
[28]P. Mishra, M. Hill, and P. Glynne-Jones, Biomicrofluidics **8**, 34109 (2014).
[29]Y. Sun, X. Yang, J. Zhou, S. Qiu, J. Fan, and Y. Xu, J. Cancer Res. Clin. **137**, 1151 (2011).
[30]E. Lianidou and A. Markou, Clin. Chem. **57**, 1242 (2011).
[31]T. Fehm, V. Muller, C. Alix-Panabieres, and K. Pantel, Breast Cancer Res. **10**(Suppl. 1), 1 (2008).
[32]B. Casavant, R. Mosher, J. W. Warrick, L. Maccoux, S. Berry, J. Becker, V. Chen, J. Lang, D. McNeel, and D. Beebe, Methods **64**, 137 (2013).
[33]M. Miller, G. Doyle, and L. Terstappen, J. Oncol. **2010**, 617421 (2010).
[34]P. Cen, X. Ni, J. Yang, D. Graham, and M. Li, Biochim. Biophys. Acta **1826**, 350 (2013).
[35]K. Hyun, J. Kim, H. Gwak, and H. Jung, Analyst **141**, 382 (2016).
[36]A. Meunier, J. Hernandez-Castro, K. Turner, K. Li, T. Veres, and D. Juncker, Anal. Chem. **88**, 8510 (2016).
[37]Y. Xin, J. Kim, M. Ni, Y. Wei, H. Okamoto, J. Lee, C. Adler, K. Cavino, A. Murphy, G. Yancopoulos, H. Lin, and J. Gromada, Proc. Natl. Acad. Sci. U. S. A.**113**, 3293 (2016).
[38]A. Reece, B. Xia, Z. Jiang, B. Noren, R. McBride, and J. Oakey, Curr. Opin. Biotechnol. **40**, 90 (2016).
[39]D. Hümmer, F. Kurth, N. Naredi-Rainer, and P. S. Dittrich, Lab Chip **16**, 447 (2016).
[40]A. Grünberger, W. Wiechert, and D. Kohlheyer, Curr. Opin. Biotechnol. **29**, 15 (2014).
[41]G. Du, Q. Fang, and J. den Toonder, Anal. Chim. Acta **903**, 36 (2016).
[42]S. Hosic, S. Murthy, and A. Koppes, Anal. Chem. **88**, 354 (2016).
[43]X. Mu, W. Zheng, J. Sun, W. Zhang, and X. Jiang, Small **9**, 9 (2013).
[44]Y. Liu and H. Lu, Curr. Opin. Biotechnol. **39**, 215 (2016).
[45]K. Love, S. Bagh, J. Choi, and J. Love, Trends Biotechnol. **31**, 280 (2013).
[46]L. Yu, S. Ng, Y. Xu, H. Dong, Y. Wang, and C. Li, Lab Chip **13**, 3163 (2013).
[47]T. Kaminski, O. Scheler, and P. Garstecki, Lab Chip **16**, 2168 (2016).
[48]E. Basova and F. Foret, Analyst **140**, 22 (2015).
[49]F. Coumans, G. Dalum, M. Beck, and L. Terstappen, PLoS One **8**, e61770 (2013).
[50]Y. Kim, G. Koo, J. Lee, H. Moon, D. Kim, D. Lee, J. Lee, J. Oh, J. Park, M. Kim, H. Woo, S. Kim, P. Kang, W. Choi, T. Sim, W. Park, J. Lee, and Y. Kim, Biomaterials **35**, 7501 (2014).
[51]E. Kim, J. Lee, B. Kim, S. Rhim, J. Kim, K. Kim, S. Jung, P. Park, H. Park, J. Lee, and B. Jeon, Anal. Biochem. **440**, 114 (2013).
[52]Y. Cheng, X. Ye, Z. Ma, S. Xie, and W. Wang, Biomicrofluidics **10**, 014118 (2016).
[53]Y. Tang, J. Shi, S. Li, L. Wang, Y. E. Cayre, and Y. Chen, Sci. Rep. **4**, 6052 (2014).
[54]S. Lee, K. Hyun, S. Kim, J. Kang, and H. Jung, J. Chromatogr. A **1377**, 100 (2015).
[55]Y. Cheng, Y. Wang, Z. Ma, W. Wang, and X. Ye, Lab Chip **16**, 4517 (2016).
[56]A. Bhagat, S. Kuntaegowdanahalli, and I. Papautsky, Lab Chip **8**, 1906 (2008).
[57]N. Xiang and Z. Ni, Biomed. Microdevices **17**, 110 (2015).

[58]S. Nathamgari, B. Dong, F. Zhou, W. Kang, J. Giraldovela, T. McGuire, R. McNaughton, C. Sun, J. Kessler, and H. Espinosa, Lab Chip **15**, 4591 (2015).
[59]P. Tallapragada, N. Hasabnis, K. Katuri, S. Sudarsanam, K. Joshi, and M. Ramasubramanian, J. Micromech. Microeng. **25**, 084013 (2015).
[60]M. Warkiani, G. Guan, K. Luan, W. Lee, A. Bhagat, P. Chaudhuri, D. Tan, W. Lim, S. Lee, P. Chen, C. Lim, and J. Han, Lab Chip **14**, 128 (2014).
[61]R. Devendra and G. Drazer, Anal. Chem. **84**, 10621 (2012).
[62]Z. Liu, F. Huang, J. Du, W. Shu, H. Feng, X. Xu, and Y. Chen, Biomicrofluidics **7**, 011801 (2013).
[63]A. J. Laki, L. Botzheim, K. Iván, V. Tamási, and P. Civera, BioNanoScience **5**, 48 (2015).
[64]H. Okano, T. Konishi, T. Suzuki, T. Suzuki, S. Ariyasu, S. Aoki, R. Abe, and M. Hayase, Biomed. Microdevices **17**, 59 (2015).
[65]K. Loutherback, J. D'Silva, L. Liu, A. Wu, R. H. Austin, and J. Sturm, AIP Adv. **2**, 42107 (2012).
[66]Z. Liu, W. Zhang, F. Huang, H. Feng, W. Shu, X. Xu, and Y. Chen, Biosen. Bioelectron. **47**, 113 (2013).
[67]N. Lewpiriyawong, C. Yang, and Y. Lam, Electrophoresis **31**, 2622 (2010).
[68]M. Alshareef, N. Metrakos, P. E. Juarez, F. Azer, F. Yang, X. Yang, and G. Wang, Biomicrofluidics **7**, 11803 (2013).
[69]C. Huang, S. Santana, H. Liu, N. Bander, B. Hawkins, and B. Kirby, Electrophoresis **34**, 2970 (2013).
[70]M. Sun, P. Agarwal, S. Zhao, Y. Zhao, X. Lu, and X. He, Anal. Chem. **88**, 8264 (2016).
[71]P. Benhal, J. Chase, P. Gaynor, B. Oback, and W. Wang, Lab Chip **14**, 2717 (2014).
[72]L. Huang, L. Tu, X. Zeng, L. Mi, X. Li, and W. Wang, Micromachines **7**, 141 (2016).
[73]H. Song, J. Rosano, Y. Wang, C. Garson, B. Prabhakarpandian, K. Pant, G. Klarmann, A. Perantoni, L. Alvarez, and E. Lai, Lab Chip **15**, 1320 (2015).
[74]P. Zellner, T. Shake, Y. Hosseini, D. Nakidde, M. Riquelme, A. Sahari, A. Pruden, B. Behkam, and M. Agah, Electrophoresis **36**, 277 (2015).
[75]A. Lalonde, M. F. Romero-Creel, and B. H. Lapizco-Encinas, Electrophoresis **36**, 1479 (2015).
[76]M. Mohammadi, M. Zare, H. Madadi, J. Sellares, and J. Casals-Terre, Anal. Bional. Chem. **408**, 5285 (2016).
[77]E. Elvington, A. Salmanzadeh, M. Stremler, and R. Davalos, J. Visualized Exp. **79**, e50634 (2013).
[78]C. Jen, N. A. Maslov, H. Shih, Y. Lee, and F. Hsiao, Microsyst. Technol. **18**, 1879 (2012).
[79]A. LaLonde, M. Romero-Creel, M. Saucedo-Espinosa, and B. Lapizco-Encinas, Biomicrofluidics **9**, 64113 (2015).
[80]R. Pethig, Biomicrofluidics **4**, 022811 (2010).
[81]Y. Hou, Z. Wang, Y. Hu, D. Li, and R. Qiu, J. Opt. **18**, 035401 (2016).
[82]O. Brzobohatý, V. Karásek, M. Šiler, L. Chvátal, T. Čižmár, and P. Zemánek, Nat. Photonics **7**, 123 (2013).
[83]B. Landenberger, H. Höfemann, S. Wadle, and A. Rohrbach, Lab Chip **12**, 3177 (2012).
[84]B. Ma, B. Yao, F. Peng, S. Yan, M. Lei, and R. Rupp, J. Opt. **14**, 105702 (2012).
[85]H. Chen and D. Sun, IEEE Trans. Rob. **28**, 1069 (2012).
[86]X. Yan and D. Sun, IEEE Trans. Control Syst. Technol. **23**, 176 (2015).
[87]X. Wang, S. X. Chen, M. Kong, Z. K. Wang, K. Costa, R. Li, and D. Sun, Lab Chip **11**, 3656 (2011).
[88]X. Ding, S. Lin, M. I. Lapsley, S. Li, X. Guo, C. Chan, I. Chiang, L. Wang, J. McCoy, and T. Huang, Lab Chip **12**, 4228 (2012).
[89]A. Yang and H. Soh, Anal. Chem. **84**, 10756 (2012).
[90]L. Ren, Y. Chen, P. Li, Z. Mao, P. Huang, J. Rufo, F. Guo, L. Wang, J. McCoy, and S. Levine, Lab Chip **15**, 3870 (2015).
[91]C. Devendran, N. Gunasekara, D. Collins, and A. Neild, RSC Adv. **6**, 5856 (2016).
[92]X. Ding, Z. Peng, S. Lin, M. Geri, S. Li, P. Li, Y. Chen, M. Dao, S. Suresh, and T. Huang, Proc. Natl. Acad. Sci. U. S. A. **111**, 12992 (2014).
[93]C. Mu, Z. Zhang, M. Lin, Z. Dai, and X. Cao, Sens. Actuator, B **215**, 77 (2015).
[94]Y. Chen, S. Li, Y. Gu, P. Li, X. Ding, L. Wang, J. McCoy, S. Levine, and T. Huang, Lab Chip **14**, 924 (2014).
[95]J. Behrens, S. Langelier, A. Rezk, G. Lindner, L. Yeo, and J. Friend, Lab Chip **15**, 43 (2015).
[96]P. Li, Z. Mao, Z. Peng, L. Zhou, Y. Chen, P. Huang, C. I. Truica, J. Drabick, W. El-Deiry, M. Dao, S. Suresh, and T. Huang, Proc. Natl. Acad. Sci. U. S. A. **112**, 4970 (2015).
[97]D. J. Collins, A. Neild, and Y. Ai, Lab Chip **16**, 471 (2016).
[98]F. Thege, T. Lannin, T. Saha, S. Tsai, M. Kochman, M. Hollingsworth, A. Rhim, and B. Kirby, Lab Chip **14**, 1775 (2014).
[99]M. Tang, C. Wen, L. Wu, S. Hong, J. Hu, C. Xu, D. Pang, and Z. Zhang, Lab Chip **16**, 1214 (2016).
[100]K. Andree, A. Barradas, A. Nguyen, A. Mentink, I. Stojanovic, J. Baggerman, J. van Dalum, C. van Rijn, and L. Terstappen, ACS Appl. Mater. Interface **8**, 14349 (2016).
[101]A. Ring, N. Mineyev, W. Zhu, E. Park, C. Lomas, V. Punj, M. Yu, D. Barrak, V. Forte, T. Porras, D. Tripathy, and J. Lang, Oncotarget **6**, 44623 (2015).
[102]R. He, L. Zhao, Y. Liu, N. Zhang, B. Cheng, Z. He, B. Cai, S. Li, W. Liu, S. Guo, Y. Chen, B. Xiong, and X. Zhao, Biomed. Microdevices **15**, 617 (2013).
[103]K. Hyun, T. Lee, and H. Jung, Anal. Chem. **85**, 4439 (2013).
[104]O. Yassine, C. P. Gooneratne, D. Abu Smara, F. Li, H. Mohammed, J. Merzaban, and J. Kosel, Biomicrofluidics **8**, 34114 (2014).
[105]P. Chen, Y. Huang, G. Bhave, K. Hoshino, and X. Zhang, Ann. Biomed. Eng. **44**, 1710 (2016).
[106]Y. Lu, H. Liang, T. Yu, J. Xie, S. Chen, H. Dong, P. J. Sinko, S. Lian, J. Xu, J. Wang, S. Yu, J. Shao, B. Yuan, L. Wang, and L. Jia, Cancer **121**, 3036 (2015).
[107]P. Chen, Y. Huang, K. Hoshino, and X. Zhang, Lab Chip **14**, 446 (2014).
[108]A. Samanta, N. Modak, A. Datta, and R. Ganguly, Microfluid. Nanofluid. **20**, 87 (2016).
[109]M. Magbanua and J. Park, Methods **64**, 114 (2013).
[110]Y. Huang, P. Chen, C. Wu, K. Hoshino, K. Sokolov, N. Lane, H. Liu, M. Huebschman, E. Frenkel, and J. Zhang, Sci. Rep. **5**, 16047 (2015).
[111]B. Dura, Y. Liu, and J. Voldman, Lab Chip **14**, 2783 (2014).
[112]R. Ramji, V. Wong, A. Chavali, L. Gearhart, and K. Miller-Jensen, Integr. Biol. **7**, 998 (2015).

[113]K. Zhang, C. Chou, X. Xia, M. Hung, and L. Qin, Proc. Natl. Acad. Sci. U. S. A. **111**, 2948 (2014).
[114]A. Benavente-Babace, D. Gallego-Pérez, D. J. Hansford, S. Arana, E. Pérez-Lorenzo, and M. Mujika, Biosen. Bioelectron. **61**, 298 (2014).
[115]T. Arakawa, M. Noguchi, K. Sumitomo, Y. Yamaguchi, and S. Shoji, Biomicrofluidics **5**, 014114 (2011).
[116]T. Yeo, S. Tan, C. Lim, D. Lau, Y. Chua, S. Krisna, G. Iyer, G. Tan, T. Lim, D. Tan, W. Lim, and C. Lim, Sci. Rep. **6**, 22076 (2016).
[117]D. Jin, B. Deng, J. Li, W. Cai, L. Tu, J. Chen, Q. Wu, and W. Wang, Biomicrofluidics **9**, 14101 (2015).
[118]L. Mi, L. Huang, J. Li, G. Xu, Q. Wu, and W. Wang, Lab Chip **16**, 4507 (2016).
[119]B. Plouffe, S. Murthy, and L. Lewis, Rep. Prog. Phys. **78**, 16601 (2015).
[120]S. Arai, M. Okochi, K. Shimizu, T. Hanai, and H. Honda, Biotechnol. Bioeng. **113**, 112 (2016).
[121]N. Alon, T. Havdala, H. Skaat, K. Baranes, M. Marcus, I. Levy, S. Margel, A. Sharoni, and O. Shefi, Lab Chip **15**, 2030 (2015).
[122]H. Esmaeilsabzali, T. Beischlag, M. Cox, A. M. Parameswaran, and E. Park, Biotehcnol. Adv. **31**, 1063 (2013).
[123]W. Liu, N. Dechev, I. Foulds, R. Burke, A. Parameswaran, and E. Park, Lab Chip **9**, 2381 (2009).
[124]H. Esmaeilsabzali, T. Beischlag, M. Cox, N. Dechev, A. Parameswaran, and E. Park, Biomed. Microdevices **18**, 22 (2016).
[125]M. Bryan, K. Smith, M. Real, M. Bashir, P. Fry, P. Fischer, M. Im, T. Schrefl, and J. Haycock, IEEE Magn. Lett. **1**, 1500104 (2010).
[126]S. Rampini, P. Li, and G. Lee, Lab Chip **16**, 3645 (2016).
[127]G. Vieira, T. Henighan, A. Chen, A. Hauser, F. Yang, J. Chalmers, and R. Soorykumar, Phys. Rev. Lett. **103**, 128101 (2009).
[128]M. Donolato, A. Torti, N. Kostesha, M. Deryabina, E. Sogne, P. Vavassori, M. Hansen, and R. Bertacco, Lab Chip **11**, 2976 (2011).
[129]Y. Wang, Y. Zhang, S. Zhang, Z. Zhao, and C. Min, Anal. Methods **8**, 3698 (2016).
[130]J. Zakrisson, B. Singh, P. Svenmarker, K. Wiklund, H. Zhang, S. Hakobyan, M. Ramstedt, and M. Andersson, Langmuir **32**, 4521 (2016).
[131]X. Wang, S. Chen, Y. T. Chow, C. Kong, R. A. Li, and D. Sun, RSC Adv. **3**, 23589 (2013).
[132]M. Xie, Y. Wang, G. Feng, and D. Sun, IEEE/ASME Trans. Mechatronics **20**, 2242 (2015).
[133]M. Zhong, X. Wei, J. Zhou, Z. Wang, and Y. Li, Nat. Commun. **4**, 1768 (2013).
[134]X. Li, C. Cheah, S. Hu, and D. Sun, Automatica **49**, 1614 (2013).
[135]C. Liberale, G. Cojoc, F. Bragheri, P. Minzioni, G. Perozziello, R. La Rocca, L. Ferrara, V. Rajamanickam, E. Di Fabrizio, and I. Cristiani, Sci. Rep. **3**, 1258 (2013).
[136]M. Yevnin, D. Kasimov, Y. Gluckman, Y. Ebenstein, and Y. Roichman, Biomed. Opt. Express **4**, 2087 (2013).
[137]M. Werner, F. Merenda, J. Piguet, R. Salathé, and H. Vogel, Lab Chip **11**, 2432 (2011).
[138]T. Kodama, T. Osaki, R. Kawano, K. Kamiya, N. Miki, and S. Takeuchi, Biosen. Bioelectron. **47**, 206 (2013).
[139]T. Tanaka, F. Mizutani, and T. Yasukawa, Electrochemistry **84**, 361 (2016).
[140]T. Hunt and R. Westervelt, Biomed. Microdevices **8**, 227 (2006).
[141]X. Guo and R. Zhu, Sci. Rep. **6**, 31392 (2016).
[142]S. Han, Y. Joo, and K. Han, Analyst **138**, 1529 (2013).
[143]F. Fabbri, S. Carloni, W. Zoli, P. Ulivi, G. Gallerani, P. Fici, E. Chiadini, A. Passardi, G. Frassineti, and A. Ragazzini, Cancer Lett. **335**, 225 (2013).
[144]M. Suzuki, R. Nakano, and Y. Iribe, Electrochemistry **84**, 319 (2016).
[145]S. Kim, T. Yamamoto, D. Fourmy, and T. Fujii, Small **7**, 3239 (2011).
[146]M. Sen, K. Ino, J. Ramon-Azcon, H. Shiku, and T. Matsue, Lab Chip **13**, 3650 (2013).
[147]S. Lin, X. Mao, and T. Huang, Lab Chip **12**, 2766 (2012).
[148]X. Ding, S. Lin, B. Kiraly, H. Yue, S. Li, I. Chiang, J. Shi, S. Benkovic, and T. Huang, Proc. Natl. Acad. Sci. U. S. A. **109**, 11105 (2012).
[149]D. Ahmed, A. Ozcelik, N. Bojanala, N. Nama, A. Upadhyay, Y. Chen, W. Hanna-Rose, and T. Huang, Nat. Commun. **7**, 11085 (2016).
[150]D. J. Collins, B. Morahan, J. Garcia-Bustos, C. Doerig, M. Plebanski, and A. Neild, Nat. Commun. **6**, 8686 (2015).
[151]F. Guo, Z. Mao, Y. Chen, Z. Xie, J. Lata, P. Li, L. Ren, J. Liu, J. Yang, M. Dao, S. Suresh, and T. Huang, Proc. Natl. Acad. Sci. U. S. A. **113**, 1522 (2016).
[152]J. Hwang, D. Cheon, H. Shin, H. Kim, and J. Lee, Appl. Phys. Lett. **106**, 183704 (2015).
[153]M. Azarpeyvand and M. Azarpeyvand, J. Sound Vib. **332**, 2338 (2013).
[154]J. Hwang, J. Kim, J. Park, C. Lee, H. Jung, J. Lee, and K. Shung, Sci. Rep. **6**, 27238 (2016).
[155]J. Hwang, C. Lee, K. Lam, H. Kim, J. Lee, and K. Shung, IEEE Trans. Ultrason. Ferroelectr. **61**, 399 (2014).
[156]A. Mongersun, I. Smeenk, G. Pratx, P. Asuri, and P. Abbyad, Anal. Chem. **88**, 3257 (2016).
[157]F. Ben, M. Turetta, G. Celetti, A. Piruska, M. Bulfoni, D. Cesselli, W. Huck, and G. Scoles, Angew. Chem. Int. Ed. **55**, 8581 (2016).
[158]R. Weinmeister, E. Freeman, I. Eperon, A. Stuart, and A. Hudson, ACS Nano **9**, 9718 (2015).
[159]J. Wang, L. Shang, Y. Cheng, H. Ding, Y. Zhao, and Z. Gu, Small **11**, 3890 (2015).
[160]X. Li, F. Li, J.Yang, H. Kinoshita, M. Oishi, and M. Oshima, Chem. Eng. Sci. **69**, 340 (2012).
[161]M. Pollack, A. Shenderov, and R. Fair, Lab Chip **2**, 96 (2002).
[162]Y. Gai, J. W. Khor, and S. K. Y. Tang, Lab Chip **16**, 3058 (2016).
[163]M. Pan, L. Rosenfeld, M. Kim, M. Xu, E. Lin, R. Derda, and S. K. Y. Tang, ACS Appl. Mater. Interface **6**, 21446 (2014).
[164]A. Klein, L. Mazutis, I. Akartuna, N. Tallapragada, A. Veres, V. Li, L. Peshkin, D. Weitz, and M. Kirschner, Cell **161**, 1187 (2015).
[165]S. Mashaghi, A. Abbaspourrad, D. Weitz, and A. van Oijen, TrAC-Trend Anal. Chem. **82**, 118 (2016).
[166]M. Guo, A. Rotem, J. Heyman, and D. Weitz, Lab Chip **12**, 2146 (2012).