Chapter 1
Advances of Single-Cell Analysis on Microfluidics

Qiushi Huang and Jin-Ming Lin

Abstract The advances of microfluidic technologies have promoted researchers to study the inherent heterogeneity of single cells in cell populations. This will be helpful in the acknowledgment of major disease and invention of personalized medicine. Different microfluidic approaches provide varieties of functions in the process of single-cell analysis. In this chapter, we introduce decades of the history in single-cell analysis and give an outline of the mechanisms of various microfluidic-based approaches for cell sorting, single-cell isolation, and single-cell lysis.

Keywords Microfluidics development · Single-cell analysis · Mechanisms

1.1 Introduction

Cells, as the basic unit of life, are the cornerstone of biology. Analysis of life's most basic units is able to provide insides into some of the most fundamental processes in life sciences. This is so-called single-cell analysis which cross-links analytical chemistry, classical cell biology, genomics, and proteomics. More and more scientists have focused on the single-cell analysis. As the demand for studying single cells expands, they will require delicate analytical tools to obtain and account for the results. Just as the development of cell theory powered advances in biology, it is clear that single-cell analysis will open new prospects for scientists to explore.

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1.1.1 The Origin of Single-Cell Analysis

The advance of new technologies promotes discovery of new biological knowledge. Till now, the advanced tools help to acquire increasingly refined information of abundant biomolecules within cells which lead to rapid growth of molecular and cellular assays. However, the assay results of average signals from many cells are difficult to explain in many fields such as hematology, stem cell biology, tissue engineering, and cancer biology. This can be attributed to different time dynamics of a sample and population heterogeneity within cells. Thus, measures of the cell population shall be misleading which necessitates single-cell methods in molecular biology.

Single-cell heterogeneity in a population is a common focus in single-cell analysis and differs from the traditional assays of average levels of molecules within the population. Commonly, technologies for molecular-level information from a cell population are not suitable for complex and heterogeneous cell samples. Therefore, it is important to apply the right tool for better data collection. In a standard molecular assay, all cells are disposed under the same condition and then analyzed with a substantive test. The steps can be accessible but may mistakenly result in the average distribution of behavior among all the cells in the sample. For instance, time-response signals of a single cell shall be covered up under cell populations due to the different time period of each cell (Fig. 1.1a). These signals are important because cells even with identical genes respond dynamical differently to stimuli due to epigenetic differences and randomness of intracellular signaling [1]. Besides, informational cells in complex samples are often among cell populations. In this case, informational cells' response to stimuli will be mixed with other cells' response which is weak and scarce. Such a measuring result cannot represent informational cells at single-cell resolution (Fig. 1.1b). Considering bimodal expression levels when cells respond dynamically to extracellular conditions [2], a bulk measurement of this population will output an average value that is not representative of either subpopulation (Fig. 1.1c). Another case is those cells of interest are a small percentage of the population; a bulk measurement of this population will lose the key information from cells of interest (Fig. 1.1d). As a result, only by measuring the character of single cells will the real heterogeneity and behavior of the population be analyzed.

Cell heterogeneity in population has promoted understanding in the fields of systems biology, stem cell biology, and cancer biology. Since the development of single-cell analysis techniques, these fields above have been benefited at the same time [3]. The heterogeneity of tumors has already been known in cancer biology [4]. And it is possible to infer tumor progression from genomic heterogeneity [5]. This condition has gained immense interest and insights. Using single-cell techniques, dynamic proteomics has been applied to measure the single-cell response to drug stimuli [6]. Besides, single-cell gene expression techniques have been developed for the studies of stem cells [7]. Using single-cell transcriptome analysis,
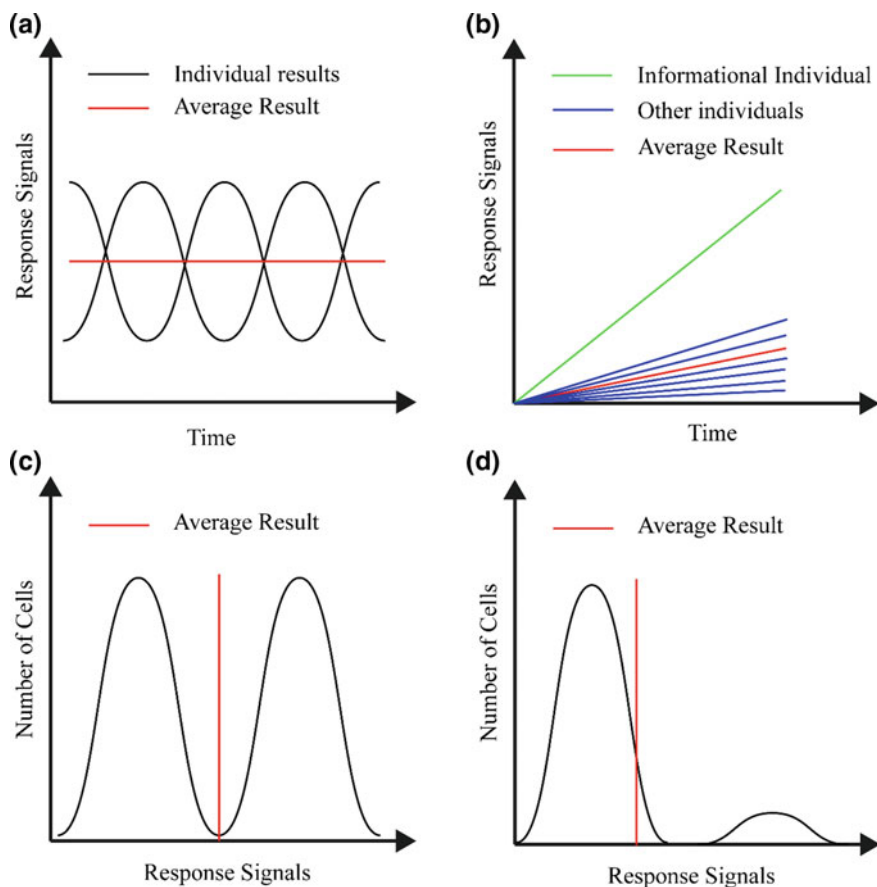
Fig. 1.1 Cell heterogeneity in population. **a** Time-response signals of single cells. **b** Informational cells' respond to stimuli mix with other cells' respond which is weak and scarce. **c** Subpopulations in bimodal expressions. **d** Rare cell populations are not accurately expressed by average assays

highly heterogeneous genetic expression profiles can be observed which is hardly to achieve by normal approaches.

Invention of the microfluidic technique has greatly promoted single-cell analysis. Traditional detection systems on multi-cells can be applied at single-cell resolution through this technique as well as isolating single populations from complex samples. Due to the development of microfabrication techniques, traditional analysis platforms have been miniaturized into micrometer scale which is so-called microfluidic lab-on-a-chip platforms. These tools support precise control of mechanical and chemical access [8] and are able to collect highly quantifiable single-cell data from high-throughput assays [9]. These miniaturized systems focus on evaluating of cell–cell interactions [10] and cell–substrates interactions [11]. Conventional macroscale platforms cannot achieve simulation in vivo microscale environments,

which microfluidic techniques can easily realize [12]. To analyze practical samples with complex components such as blood and cancer tissue, the high-throughput method is needed. Microfluidic meets the requirement of assigning precise positions in arrays or positioning in flow. Single-cell manipulation such as mechanical trapping [13], inertial isolation [14], dielectric isolation [15], and acoustic isolation [16] has been developed well suited for the single-cell analysis.

1.1.2 *Development of Single-Cell Approaches*

Single-cell analysis can be traced back to the year 1965, (Table 1.1) when Matioli et al. [17] firstly reported the isolation and direct observation of hemoglobin in single erythrocytes. Then, quantities of conventional techniques have been developed such as microthin layer chromatography (mTLC), gas chromatography–mass spectrometry (GC-MS), high-performance liquid chromatography–electrochemical (HPLC-EC) detection, and radioactive labeling [18–21]. However, these techniques are commonly not sensitive enough or limited in multiple components test thus have not been widely developed. mTLC requires several cells for sufficient result which is not strictly single-cell resolution. GC-MS is only applied for volatile compounds analysis. While target molecules are nonvolatile, derivatization will be required which limits the application. Results from HPLC-EC are highly related to

Table 1.1 Important works in the development of single-cell analysis

Year	Research	Reference
1965	Isolation and direct observation of haemoglobin in single erythrocytes	Matioli GT et al. Science 150(3705):1824–1826
1976	Patch clamping	Neher E et al. Nature 260(5554):799–802
1987	Open tubular liquid chromatography for the electrochemical analysis of single neurons	Kennedy RT et al. Mikrochimica Acta 2(1–3):37–4
1988	Single-cell capillary zone electrophoresis	Wallingford RA et al. Analytical Chemistry 60 (18):1972–1975
2000	Single-cell proteomics	Zhang Z et al. Analytical Chemistry 72(2):318–322
	Valves	Unger MA et al. Science 288(5463):113–116
2005	Droplets	Utada AS et al. Science 308(5721):537–541
2009	Live single-cell MS	Masujima T. Analytical Sciences the International Journal of the Japan Society for Analytical Chemistry, 2009, 25(8):953
2015	Single-cell MALDI-MS	Ong TH et al. Analytical Chemistry 87(14):7036–7042
2018	Live single-cell extractor	Mao S et al. Angewandte Chemie International Edition 57(1):236–240

the cell size and concentration of the determinant which is hardly to control. Radioactive labeling is limited for one kind of compound detection at one time, and the unknown compound is not suitable. The development of flow cytometry has been widely applied in cytobiology [22]. When the sample is complex that contains multiple populations of cells, flow cytometry is able to detect the physical and chemical parameters of single cells. Parameters such as size, volume, quantity, and even the content of proteins and nucleic acids can be collected at high throughput. However, the flow cytometry also has limitations in the single-cell analysis that limits the universality, such as expensive instrument costs, complex cell surface markers, increased sample pre-treatment time and reduced cell viability, as well as sample contamination risk. Patch clamping was developed by Neher and Sakmann in 1976 [23]. This technique was applied for the discovering of the function of single ion channels in cells which has won the Nobel Prize in Physiology or Medicine in 1991. The high sensitivity and high spatial resolution of the patch clamping have great potential for the study of the rapid reaction kinetics in the ultra-microenvironment of single-cell organisms.

Single-cell analysis meets the formidable challenge that requires methods that are more sensitive, more selective, more quantitative, more informative, and can detect more analytes at the same time.

In 1987, Kennedy et al. [24] firstly developed open-tubular liquid chromatography for the electrochemical analysis of single neurons. In this approach, individual neurons from the subesophageal ganglia of Helix aspersa were analyzed. The putative neurotransmitters, dopamine and serotonin, and their precursor amino acids, tyrosine and tryptophan, were identified and quantified. However, the device and micro-operation techniques in this method are complex, and it is difficult to analyze small single cells. Subsequent development of this technique has been restricted.

Capillary electrophoresis (CE) can meet the requirements of single-cell analysis on small volume sampling, high sensitivity, good selectivity, multi-component analysis, and fast response. Ewing's group [25] has developed single-cell capillary zone electrophoresis (CZE) by in vivo analysis dopamine from a single nerve cell. Miniaturization works of the device were still demanded for the feasibility of mammalian cells which are much smaller than neurons.

Sweedler's group [26–29] has conducted research in single-cell analysis and focus on the development of analytical methods for assaying complex microenvironments, including CE, laser-based detectors, and MALDI sampling techniques. Their utilizing of MALDI-MS has achieved the detection of the spatial distribution of neuropeptide in different parts of nerve cells. Other studies include the metabolism, dynamic release of neuropeptides and classical transmitters in a cell-specific manner.

Dovichi's group has proposed single-cell proteomics in 2000 [30]. They are trying to develop the tools for studying the proteome with two-dimensional CE, coupled with either laser-induced fluorescence (LIF), or MS for detection. It seems the group's long-term goal is to study protein expression in single cells and to determine how protein expression changes across a cellular population during cancer progression and during the development of an embryo. Till now, they have

acquired important achievements [31–33]. However, for the project of single-cell proteomics, it is still the beginning.

Microfluidic technologies in the single-cell analysis have been widely developed since the twenty-first century [34–36]. The microstructure of the microfluidic chip can be matched well with the single-cell volume, which is a promising single-cell analysis technology. The advantages of microfluidic techniques are listed as below:

(1) The manipulation, transmission, sampling, positioning, dissolution, reaction, separation, and detection of cells can be integrated in one microfluidic chip. The control system of micropump and microvalve can be adopted to realize the integration and automation.
(2) Microfluidic techniques supply high-throughput analysis of single cells which achieve high-speed studies of cell metabolism, gene expression, and drug screening.
(3) The reagent consumption is significantly reduced. The sample required of the microfluidic chip is measured by nL–µL, which greatly reduces the cost of experiments.
(4) High temporal resolution of microfluidic devices can be used for continuous monitoring of stimulated release of living cells and cellular molecular response processes.
(5) Closed operation environment helps to reduce the risk of sample contamination and improves cells' viability.

Zare's group has done excellent work in single-cell analysis on microfluidic [37–39]. In their previous work [40], they have developed multilayer microfluidic chips for the analysis of single cells. The microfluidic channels enabled the passive and gentle isolation of a single cell from the complex cell suspension, and integrated valves and pumps enabled the precise delivery of nanoliter volumes of reagents to the cell. Various applications were demonstrated such as cell viability analysis, ionophore-mediated intracellular Ca^{2+} flux measurements, and multistep receptor-mediated Ca^{2+} measurements. The experiments had significant improvements in reagent consumption, analysis time, and temporal resolution compared with macroscale assays.

Ramsey's group [41–43] has been devoted to the development of microfluidic technology. They are known for their pioneering efforts in demonstrating microfabricated chemical measurement devices. These devices have improved conventional laboratory measurements in microscale experiments of several orders of magnitude. The technology is presently followed by a number of research institutions and corporations around the world and is possible to be a general model for chemical and biochemical experimentation.

Our group has focused on this field as well in recent years. For cell sorting, two-dimensional ordered polystyrene microspheres based microwell arrays [44] has been developed for high-throughput single-cell analysis. In this work, rounded bottom microwell arrays (Fig. 1.2a) in poly(dimethylsiloxane) were fabricated by molding a monolayer of ordered polystyrene microspheres. Sizes of microwell were tunable in the 10–20 µm ranges which were able to capture adherent or
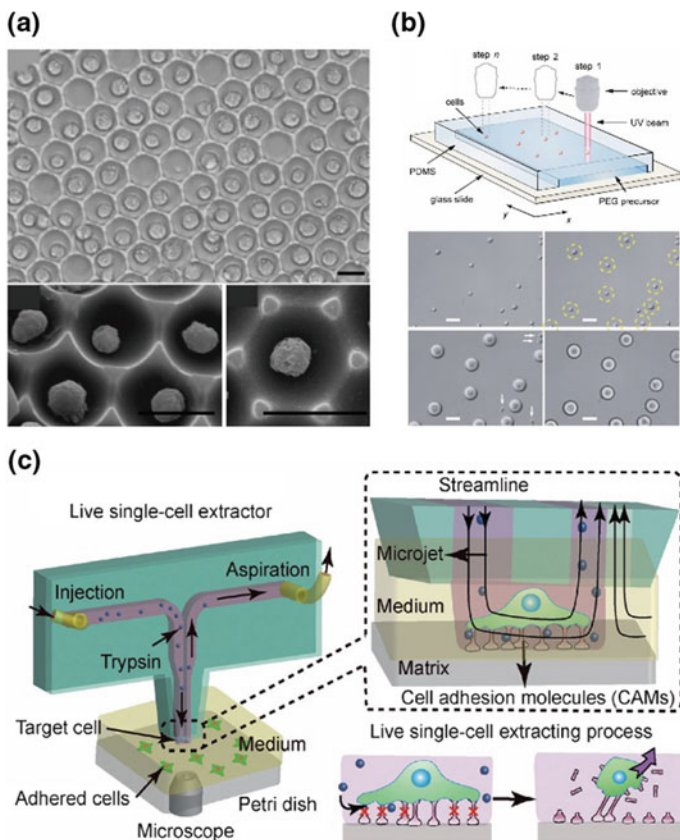
Fig. 1.2 Microfluidic enabled single-cell analysis. **a** Cell arrays on the microwells. Reprinted with permission from Ref. [44]. **b** Controlled encapsulation of single cells inside hydrogel microstructures. Reprinted with permission from Ref. [46]. **c** Microfluidic chip-based live single-cell extractor. Reprinted with permission from Ref. [11]

non-adherent cells with high efficiency. Further, designed microwell structures enable us to launch strong interactions of the target cell surface with biomolecules [45]. By coating DNA aptamer on the 3D-structured microwell, a satisfactory single-cell occupancy could be obtained.

For single-cell encapsulation, a microfluidic approach to generate hydrogel microstructures inside microchannels for controlled encapsulation of single cells was developed [46]. This approach has the capability to immobilize different phenotypes of cells inside hydrogel microstructures with different morphologies for identification (Fig. 1.2b). Recently, we reported a live single-cell extractor (Fig. 1.2c) to extract a single adhered cell in tissue culture for understanding cell heterogeneity and the connection of various single-cell behaviors [11]. By using this technique, the connection between cell adhesion strength and cell morphologies as well as that between cell adhesion strength and intracellular metabolites was explored.
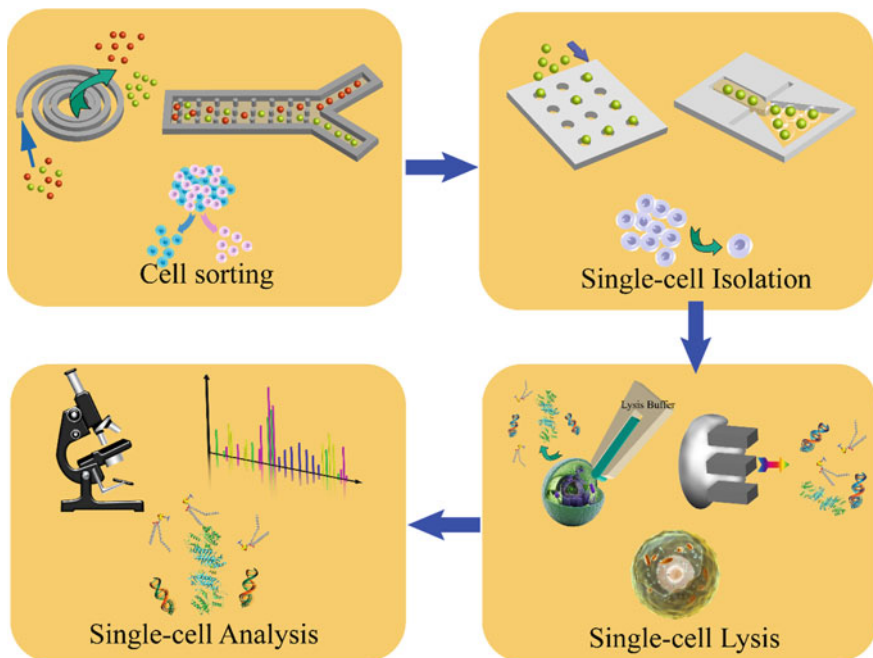
Fig. 1.3 Microfluidic enabled workflow of single-cell analysis

1.2 Mechanisms of Microfluidic Technologies in Single-Cell Analysis

As is discussed above, microfluidic devices have opened new avenues for point-of-care diagnostics due to the characteristic of fitted approximately to the size of an individual thus provided fascinating solutions to many issues in single-cell analysis. Microfluidic platforms have the advantages of portability, parallel processing, automation, and a large surface-to-volume ratio. This enables the integration of multiple liquid operating processes, such as pumping, metering, sampling, dispensing, sequential loading, and washing. These advantages make microfluidic technology more compatible and greatly reduce the labor compared with traditional laboratory technology. This part describes the various microfluidic-based approaches used for single-cell analysis which follows the workflow as shown in Fig. 1.3. Cell sorting, single-cell isolation, and single-cell lysis are involved. Up-to-date mechanisms of techniques and the pros and cons of these methods are discussed in detail. This section will be of great interest for researchers who will work in the same field and an informative tool for researchers from other fields and beginners.

1.2.1   Cell Sorting

Compared with traditional methods, microfluidic devices have inherent advantages in the cell sorting platform. Miniaturized devices reduce reagent consumption to a certain extent and increase portability. The production cost of the devices can be decreased by introducing soft lithography for standard microfabrication. The flowing cells can be controlled spatiotemporally by laminar fluid dynamics, which achieves passive and label-free cell separation. Commonly, cell sorting is achieved continuously in an enclosed microfluidic device, which minimizes the risk of sample contamination. However, several drawbacks exist as the immaturity of this technology. The throughput is a major research focus to improve compared with commercial flow cytometry. Besides, cell adhesion and clogging problems occur frequently which limit the life of the device. Till now, there are several excellent works [47–51] in the field regarding microfluidic enabled cell sorting. Here, we
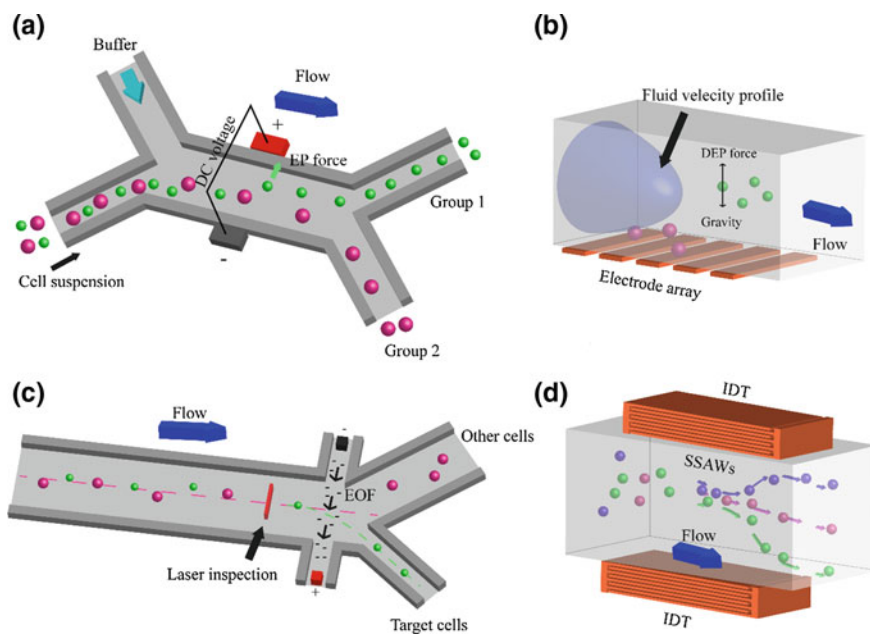

Fig. 1.4 Different microfluidic cell sorting techniques. **a** Mechanism of microfluidic cell sorting integrated with EP. A uniform electric field induces a Coulomb force on negatively charged cells, resulting in a net force toward the positive electrode. The target cells are then separated. **b** Mechanism of microfluidic cell sorting integrated with DEP. DEP force and gravity force together positions different cell types at individual regions of different flow velocity, leading to different retention time for each cell type. **c** Mechanism of microfluidic cell sorting integrated with EOF. Solvated negative ions migrate toward the positive electrode, inducing fluid flow and cell separation. The electrode charge is controlled by the signal from laser interrogation. **d** Mechanism of microfluidic cell sorting integrated with SSAWs. The standing surface acoustic waves, generated by interdigital transducers, separate cells at distinct streamlines, and the cells are separated via different outlets

introduce existing cell sorting technologies integrated on microfluidic chips that specific relevance to single-cell analysis. Different sorting mechanisms suit for different cell type's properties.

1.2.1.1 Electrical

Most cells present negative charges on the surface at the condition of neutral pH. Once a constant electric field is added (Fig. 1.4a), cells will move toward the positive electrode [52]. In cell suspension, cells are mainly driven by two forces as the Coulomb and drag forces. Different quantities of force induce different velocity of movement. Therefore, different cell types with different charge or size can be separated under this constant electric field, which is called electrophoresis (EP) sorting [53].

Dielectrophoresis (DEP) approaches [53] are used more frequently for cell sorting due to its higher specificity in dielectric properties among cell types. In DEP [54], a complex mixture of cell suspension flows through a channel while the integrated electrodes at the bottom of channel generate an upward DEP force that balances the gravity force (Fig. 1.4b). Different cell type stabilizes at an individual height in the channel. Due to the parabolic velocity profile, they will have different flow velocity for the separation. Using this technology, an alternating current will polarize the cell instead cells' inherent surface charge. Therefore, cells are not required to present surface charge and move toward or away from the area of highest electric field density. An alternating electric field is required to inflict a force on the polarized cell and the move direction is due to the electrical permeability of the cells. Under the condition that the fluid has higher permeability than cells, negative dielectrophoresis (nDEP) will drive cells moving away from the field maxima and positive dielectrophoresis (pDEP) will drive cells moving toward the field maxima. Compared to EP sorting, DEP approaches have larger cell sorting specificity, and utilizing of alternating current prevents electrochemical reactions at the electrodes and decreases detriment to cell viability.

Similar to the EP and DEP sorting mechanisms, electroosmotic enabled separation also utilizes an electric field. Electroosmotic flow (EOF) is defined as fluid flow by inducing solvated ions movement under an electric field. Then particles in the solution will accompany the fluid flow induced by migrating solvated ions. Using this technique, target cells can be picked up actively from the complex sample (Fig. 1.4c). The advantage of EOF is the accurate control of volume in microfluidics which enables precise control of small volumes of reagents and size-based cell sorting. However, electrophoretic cell movement can influence the accuracy of electroosmotic driven in this approach.
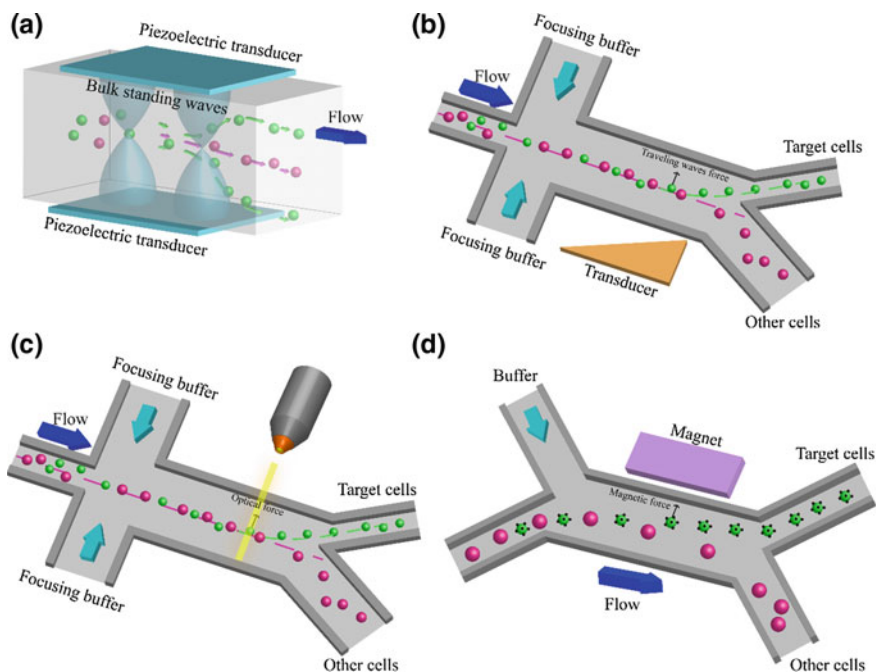
Fig. 1.5 Different microfluidic cell sorting techniques. **a** Mechanism of microfluidic cell sorting integrated with bulk standing waves. Cells separate to the wave node or antinode depending on their acoustic contrast factor. **b** Mechanism of microfluidic cell sorting integrated with traveling acoustic waves. **c** Mechanism of microfluidic cell sorting integrated with optical tweezer. A laser emitter is integrated on the microfluidic chip and the cells are deflected by the laser beam toward different outlet. **d** Mechanism of microfluidic cell sorting integrated with magnet. The device is operated under the magnetic field and the magnetic particle labeled cells are collected from different outlet with normal cells

1.2.1.2   Acoustic

Acoustic enabled cell sorting has no impact on cell viability which appears recently [55]. Acoustic separation works by inducing cell migration response to acoustically generated pressure waves. Currently, three kinds of acoustic cell sorting exist classified on the wave type as: standing surface acoustic waves, bulk standing waves, and traveling waves [47].

Standing surface acoustic waves (SSAW) form along the bottom of microfluidic channels when integrating interdigital transducers (IDTs). The IDTs are patterned on a piezoelectric substrate on the microfluidic chip which produces a longitudinal wave from the substrate and these longitudinal waves create pressure nodes for the particle separation (Fig. 1.4d). SSAWs are able to deflect particles in fluid flow independently which are flexible in separating cell populations [56].

Bulk standing waves are generated by a piezoelectric transducer in microfluidic channels when the acoustic wavelength matches the channel dimension. In this

approach, cells flowing through the channel will respond differently to the standing wave by the acoustic contrast factor, which is dependent on cell density and compressibility (Fig. 1.5a). Cells with a positive acoustic contrast factor will migrate toward the wave node, and cells with negative acoustic contrast factors will migrate toward the wave antinodes. Thus, cell sorting will be achieved by cell separation to different outlets.

Traveling waves can be used for cell sorting as well. Standing waves require wavelengths comparable to microfluidic channel width, and therefore, the sorting rate cannot be increased because the wavelengths are limited. Traveling waves overcome this limitation where acoustic waves are as a drive force similar to the electric sorting (Fig. 1.5b). The acoustic wave is generated by a transducer and the wave travels in the direction perpendicular to the flow direction, thereby deflecting cells from the fluid streamlines into the appropriate outlet channel.

Acoustic enabled cell sorting mitigates cell viability decrease which improves time and cost economy. However, similar to electrically enabled cell sorting, acoustic cell sorting requires integrated transducers. This makes fabrication and operation more complex. Examples of this technique are available from recent reviews [57, 58] for further discussion.

1.2.1.3 Optical

Light-driven particle movements can be found in 1970 when it was discovered that a focused laser could propel microparticles in a liquid [59]. Then, stable particles' trapping via a tightly focused laser was achieved [60] which was the foundation of "optical tweezers." In this technique, the optical forces form from momentum exchange between incident photons and the irradiated object. When the light rays into an object, the light's direction and magnitude will be changed due to the difference of refractive index between object and surrounding. Then, the photons' momentum will be changed associated. The particles' movements depend on their different refractive index. They will move toward the area of highest light intensity when the refractive index is higher than the surrounding fluid. In contrast, while the refractive index is lower, the particles will move to the opposite direction. Here are some reviews [61, 62] for more discussions on the light-induced forces. Optical manipulation has been utilized for cell sorting and manipulation on microfluidic chips (Fig. 1.5c). This approach has the advantage of minimally detrimental to cell viability, compared to sorting methods discussed above.

1.2.1.4 Magnetic

In magnetic enabled cell sorting, magnetic particles can be biologically combined with the target cells via a cell-specific antibody. Therefore, the target cells can be separated from the complex sample by flow through a microfluidic channel possessing a magnetic field (Fig. 1.5d). The advantages of this approach are the
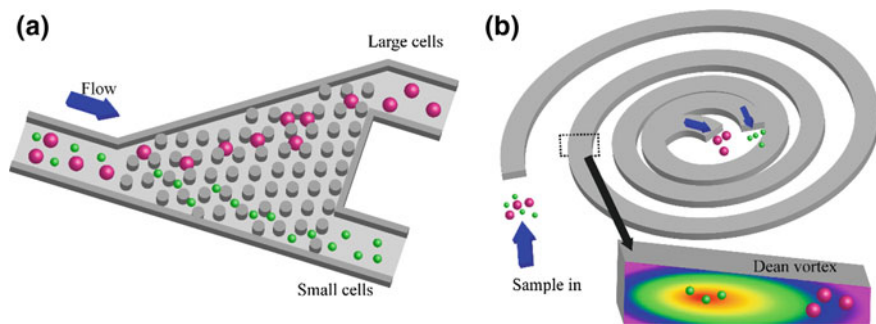
Fig. 1.6 Different microfluidic cell sorting techniques. **a** Mechanism of microfluidic cell sorting integrated with micropillars array. An array of micropillars induces cells with different radius toward different outlet. **b** Mechanism of microfluidic cell sorting based on inertial effect. A spiral channel is designed to form the vortex (Dean flow) perpendicular to the primary flow. Cells with different sizes, densities, or shapes are positioned at the vortex and the cells are separated from different outlets

simplicity and non-touching separation. The magnetic field can be induced via an integrated permanent magnet or an electromagnet for a low cost. However, applying magnetic sorting requires antibody labeling of magnetic particles on cells. This can be a drawback for several reasons. Labeling may negatively impact cells' endogenous genetic expression and is difficult to achieve in specific cells that lack of surface markers. Besides, labeling is not desirable in clinical concerns, and antibodies are quite expensive. Till now, microfluidic magnetic enabled cell sorting has been applied in many fields, and the reader can refer to several reviews [63–65].

1.2.1.5 Array of Micropillars

Here is a passive microfluidic cell sorting that uses a pillar array to induce cells' migration based on cell size. In this method, there is a critical cell size to separate different cells which are depending on the pillar design [66]. Cells with a smaller size than the critical radius flow in the direction of primary fluid. In contrast, cells bigger than the critical radius are deflected for the separation (Fig. 1.6a). This passive cell sorting approach can be advantageous due to the gentle and label-free separation. Besides, the fabrication of the device can be relatively simple. However, the limitation is that the target cells must differ from the others in size or shape, which greatly restricts its application. Readers can refer to a review for more research on this technique [67].

1.2.1.6 Inertial

When the microfluidic channel is at a suitable dimension and flow velocities, inertial effects will show up and can be utilized for cell sorting. In the curve channel, there is a typical inertial effect called Dean flow [68] which can form vortex perpendicular to the primary flow. Cells which have different size, density, or shape will respond differently to the inertial effects and have individual streamlines (Fig. 1.6b). Then, cell sorting is achieved by collecting cells from different outlets. This approach has the advantage of label-free separation and little influence on the cell viability. This can be attractive for the minimized costs, wide applicability, and maintaining most endogenous expression. Besides, this method conducts in continuous flow therefore increase the throughput of cell sorting. However, the disadvantage is obvious that the samples require to be diluted due to the cell interactions influence at high cell concentrations. Here are some reviews [69, 70] that discuss about the physics of inertial microfluidics. Our group has done relative work in this field that utilizes this principle combining with mass spectrometry, which realizes high-throughput single-cell MS analysis [71].

1.2.2 Single-Cell Isolation

Microfluidic devices offer many advantages to single-cell isolation [72]. In this technique, single-cell compartments can be miniaturized to reduce lysate dilution which is important for the analysis of low-abundance biomolecules in a single cell. Consumption of reagent volumes is minimized which decreases the costs. Most microfluidic chips are automatic and closed systems, decreasing contamination risk. In this section, a variety of microfluidic enabled single-cell isolation is introduced. The basic mechanisms of the involved methods are as the focus to discuss.

1.2.2.1 Valves

Valves can be utilized to trap objects in the microscopical pipes, which provide an easy conversion into microscale for the single-cell trapping. In microchannels, valves can be utilized to regulate fluid flow and control flow direction. Quake's group [73, 74] have developed valve on microfluidic that is so-called Quake valves, which is commonly used later in other researches. The common examples of Quake valves in microfluidic are two-layer pneumatic valves. The top layer integrated channels for the pneumatic valves. When gas passes through the channel, the barometric pressure will force the channels in the bottom layer to close, where single cells are trapped (Fig. 1.7a). The valves commonly utilize computer-controlled programs which simplify the operation but complicate device fabrication and increase the cost. The throughput of this approach is limited for the requirement of microscopy to confirm single-cell trapping. Perhaps, the combination of automatic
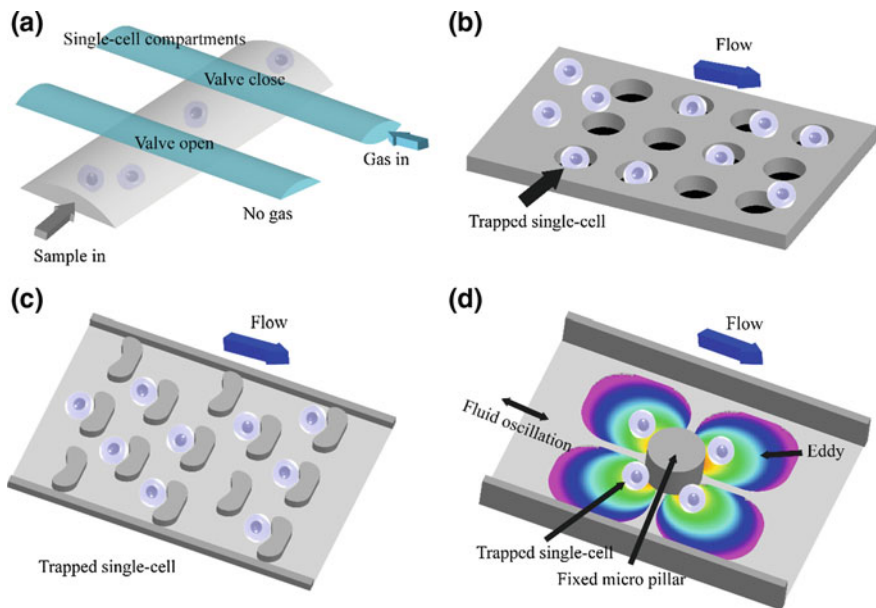
Fig. 1.7 Different microfluidic enabled single-cell isolation techniques. **a** Quake valves integrated microfluidic chip for single-cell isolation. **b** Schematic diagram of single-cell isolation based on microwells. **c** Schematic diagram of single-cell isolation based on microdams. **d** Schematic diagram of hydrodynamic single-cell isolation. An oscillating flow around a micropillar generates four surrounding eddies. Single cells are trapped at the center of eddies

feedback-controlled valves and microscopy will enable hands-off single-cell isolation and increase the throughput.

1.2.2.2 Microwells and Microdams

Single-cell isolation utilizing physical boundaries can be easily achieved by microfluidic chips. From the concept of multi-well plates for the isolating of cell groups, microwells can be used as a tool for the single-cell isolation. In this approach, microwells with suitable sizes are generated on the bottom of the channel. Single cells are trapped into individual microwells by gravity, and redundant cells will be flushed away (Fig. 1.7b). The size and shape of microwell can be adjusted to increase single-cell isolation efficiency. And the quantities of isolated single cells are depending on the scale of the microfluidic chip. However, the molecular analysis of single-cell studies is not suitable for this approach due to the non-isolated single-cell lysates. For more information about this technique, readers can refer to the previous reviews [75, 76].

Another single-cell isolation method uses physical boundaries is called microdams such as U-shaped cups to physically isolate single cells on chip (Fig. 1.7c).
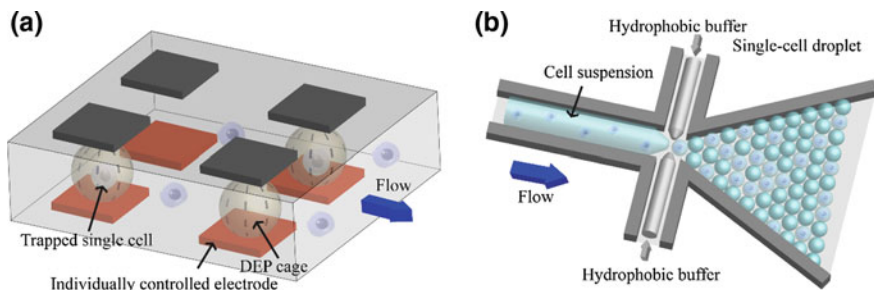
Fig. 1.8 Different microfluidic enabled single-cell isolation techniques. **a** Schematic diagram of single-cell isolation based on DEP. Individually controlled electrodes generate DEP cages to trap single cells. **b** Schematic diagram of droplets enabled single-cell isolation

The microdam is required to have cutaways allowing fluid flow through an unoccupied trap, thereby preventing clogging. This approach is beneficial because of the passive isolation and applicability for various cell sizes and shapes. However, most applications of this approach are used for single-cell culturing and transient imaging analysis, rather than single-cell lysate analysis.

1.2.2.3 Hydrodynamic

Single-cell isolation can be achieved passively by hydrodynamic mechanisms. This approach does not require complex fabrication systems thus attracting researchers to follow. In this approach, recirculating fluid flow is generated in the microfluidic channel for the cell trapping which is called eddies or vortices. It has been reported [77] that four surrounding eddies generated from an acoustic induced fluid oscillation around a micropillar can be applied to trap single cells (Fig. 1.7d). This approach is advantageous because of the passive isolation and applicability for various cell sizes and shapes. However, most application of this approach is used for single-cell culturing and transient imaging analysis, rather than single-cell lysate analysis due to the non-isolated single-cell lysates. For further discussion of hydrodynamic enabled single-cell isolation, readers can refer to the previous review [78].

1.2.2.4 Dielectrophoretic

Single-cell isolation by dielectrophoretic methods has been developed with great success. In this approach, a pair of electrodes enabling single-cell trapping is integrated into a microfluidic chip which is called DEP cage. A dielectrophoretic-based single-cell isolation system consists of a disposable cartridge which is an array of individually controllable DEP cages (Fig. 1.8a). After the process of trapping, cells are identified under a microscope and manipulated to other traps or isolated off the chip. The approach can be applied for isolating rare cancer cells from real blood

samples. However, the limitations of the approach are low throughput, inapplicability to smaller samples, and large labor costs. Besides, single cells are not strictly compartmentalized on a chip, which turns down the applicability for single-cell lysates analysis.

1.2.2.5   Droplets

Droplets recently have become a popular method in single-cell analysis. Just as its name implies, droplets are created by the incision of two immiscible fluids and can be utilized for cell encapsulation and isolation (Fig. 1.8b) [79]. This approach is especially suitable for molecular analysis from single-cell lysates. In this approach, each droplet functions as an individual chemical reactor and has no interchange of material with the others. The fabrication of systems is also simplified for easy operation. Droplet generation is achieved in a continuous flow which induces high-throughput single-cell encapsulation. Besides, the volume of each drop can be minimized into picoliter or even femtoliter. This greatly reduces the dilution of cell lysates for single-cell analysis. The single-cell capture rate is principally based on Poisson distribution, which cannot reach a hundred percent. Increasing this can be achieved by prefocusing or postsorting steps. Droplets have become a powerful platform for single-cell analysis. More discussion can be found in several focused reviews [80, 81].

1.2.3   Single-Cell Lysis

Microfluidic technology provides an ideal platform for single-cell lysis. Channels of microfluidic devices can be fabricated with special geometries and fluid flow can be controlled precisely, which allow accuracy control of single-cell lysis. The dimensions can be directly matched with the scale of single cells, which minimize the lysate dilution for better sensitivity. Most microfluidic devices are optically transparent, increasing the applicability with fluorescence detection. Close environment minimizes contamination of the sample. Till now, several approaches of microfluidic enabled single-cell lysis have been developed. Researchers often choose a suitable approach for the desired result. In this section, several popular models of microfluidic enabled single-cell lysis are discussed. And for further discussion, readers can refer to the later chapters or focused reviews [82, 83].

1.2.3.1   Chemical

Chemical lysis of cells is achieved by lysis buffer containing surfactants to solubilize lipids and proteins in the cell membrane. This process creates pores on the cell membrane and gradually induces fracture of an intact cell [84–86]. Microfluidic

devices are advantageous because of their precise control of fluid flow. Minimized lysis buffer can be consumed for the single-cell lysis which minimizes dilution (Fig. 1.9a). Although this is a simple and convenient approach in single-cell lysis, the limitation is obvious that the chemical reagent in the lysis buffer will contaminate the sample, which should be removed.

1.2.3.2 Mechanical

Mechanical lysis of cells can be achieved by tearing or puncturing cell membranes utilizing mechanical forces [87–89], such as shear stress, friction forces, or compressive stress. This approach directly breaks the cell structure and releases the target intracellular molecules (Fig. 1.9b). Mechanical enabled single-cell lysis can relatively minimize the protein damage, which is quite disturbing in other approaches. However, the cell's fragment produced by mechanical lysis makes the subsequent isolation complex.

1.2.3.3 Electrical

Electrical methods for single-cell lysis are common for generating pores on cell membranes to break the cell which are called electroporation lysis [90, 91]. In this approach, cells are disposed under an external electric field, where the potential is
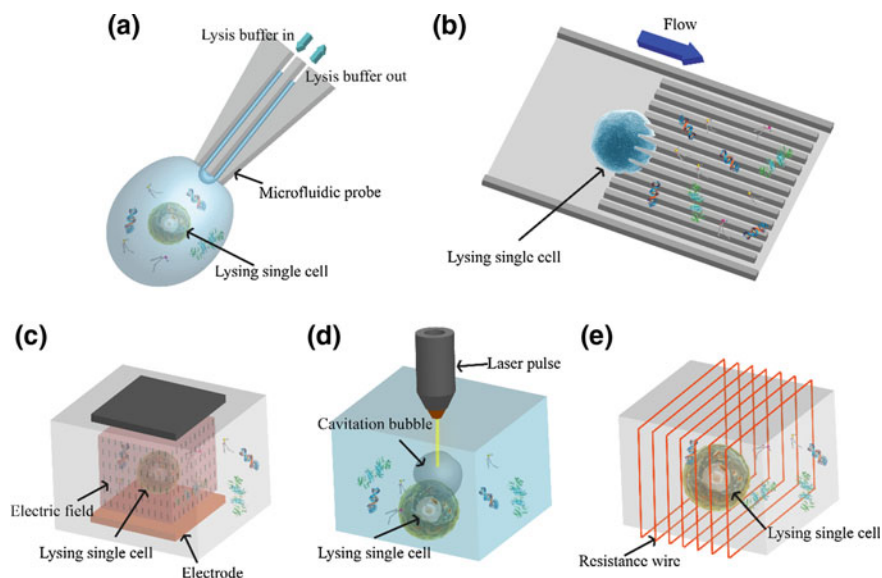

Fig. 1.9 Different microfluidic enabled single-cell lysis techniques. **a** Chemical. **b** Mechanical. **c** Electrical. **d** Optical. **e** Thermal

created through the cell membrane (also known as the transmembrane potential). Pores can be created on the cell membrane when the potential reaches a certain threshold at about 0.2-1.0 V [92]. The pores can be reversible under a mild electric field. However, while the electric field reaches to a high enough extent, the pores will become permanent and achieve complete lysis of cells (Fig. 1.9c) [93]. Commonly, reversible electroporation is utilized for small molecules' detection, and permanent electroporation is more suitable for macromolecules such DNA and proteins. Electrical enabled single-cell lysis is advantageous due to the ultra-high lysing efficiency on a millisecond level and better selectivity for different membranes by adjusting the electric field [94, 95]. However, the limitation is that the accuracy control of electrical field leads to a relatively complex operation process. And a short lifetime of electrode should be noted for the application.

1.2.3.4 Optical

Optical methods for single-cell lysis can be interpreted as the cell broken induced by fluid motion produced by a focused laser [96–98]. In this approach, a laser pulse is focused at a buffer interface around the surface of a cell. The high-energy laser will produce a localized cavitation bubble whose expansion and movement together with the induced fluid dynamic forces will help to break the cell membrane (Fig. 1.9d) [99]. Optical single-cell lysis has the advantages of high selectivity, high efficiency, and localized lysis region, which has destruction to the intracellular components [100]. Integrating the laser pulse into microfluidic chips also provides convenience controlling the time and location of the cell lysis. Besides, microchannels will restrain the over-expansion of the cavitation bubble, which is useful to control the volume [101]. However, the limitation is obvious that the complex integration of optical system greatly increases the experimental cost.

1.2.3.5 Thermal

Thermal methods for single-cell lysis can be interpreted as utilizing high temperature to denature the proteins on cell membranes which result in cell damaging for the intracellular components (Fig. 1.9e) [102, 103]. Thermal lysis is advantageous for high lysing intensity and simplicity. However, the temperature should be set carefully and controlled precisely for the existing of many heat-sensitive molecules in cells. Therefore, thermal single-cell lysis is most frequently used for parallel, on-chip PCR analysis instead of protein analysis [104, 105].

1.3   Conclusion and Outlook

Single-cell analysis is now a rapidly developing field. Various applications and future concerns will be discovered. Invention of novel microfluidic platforms will give a major push to single-cell biology. In this chapter, we have discussed the development and the mechanisms of single-cell analysis and microfluidic devices for cell sorting, single-cell isolation, and single-cell lysis.

Microfluidic enabled single-cell analysis now is a complete research field. Researchers continue to innovate and develop new techniques, which will be discussed in the later chapter. Microfabricated systems are advantageous due to little sample consumption, high cell viability, and low costs. However, the limited throughput compared with conventional flow cytometry prevents the wider adoption of microfluidic. Besides, microfluidics is not easily reusable due to clogging and cell adhesion. We expect the field of microfluidic single-cell analysis to continue growing for the widely commercialized application.

The modern science of single-cell biology is particularly fascinating. The development of detection techniques has allowed researchers to study cells in depth which are the basic units of life. Novel concepts in this field have been well established such as single-cell genomics, single-cell proteomics, and single-cell immunology which are a paradigm shift in biology. Currently, existing work in single-cell analysis always removes cells from their native environment for more convenient operation. This will lose the impact of native tissue and change cells' behavior. As such, new works often focus on the cells' original spatial context which has profound implications [106–108]. Nevertheless, these remarkable works only indicate the beginning of a new era, when revolutionary single-cell omics and innovative microfluidic approaches befall to the world.

References

1. Elowitz MB, Levine AJ, Siggia ED, Swain PS (2002) Stochastic gene expression in a single cell. Science 297(5584):1183–1186. https://doi.org/10.1126/science.1070919
2. Fiering S, Northrop JP, Nolan GP, Mattila PS, Crabtree GR, Herzenberg LA (1990) Single cell assay of a transcription factor reveals a threshold in transcription activated by signals emanating from the T-cell antigen receptor. Genes Dev 4(10):1823–1834
3. Hosic S, Murthy SK, Koppes AN (2016) Microfluidic sample preparation for single cell analysis. Anal Chem 88:354–380. https://doi.org/10.1021/acs.analchem.5b04077
4. Dexter DL, Kowalski HM, Blazar BA, Fligiel Z, Vogel R, Heppner GH (1978) Heterogeneity of tumor cells from a single mouse mammary tumor. Cancer Res 38(10): 3174–3181
5. Navin N, Krasnitz A, Rodgers L, Cook K, Meth J, Kendall J, Riggs M, Eberling Y, Troge J, Grubor V, Levy D, Lundin P, Maner S, Zetterberg A, Hicks J, Wigler M (2010) Inferring tumor progression from genomic heterogeneity. Genome Res 20(1):68–80. https://doi.org/10.1101/gr.099622.109

6. Cohen AA, Geva-Zatorsky N, Eden E, Frenkel-Morgenstern M, Issaeva I, Sigal A, Milo R, Cohen-Saidon C, Liron Y, Kam Z, Cohen L, Danon T, Perzov N, Alon U (2008) Dynamic proteomics of individual cancer cells in response to a drug. Science 322(5907):1511–1516. https://doi.org/10.1126/science.1160165

7. Zhong JF, Chen Y, Marcus JS, Scherer A, Quake SR, Taylor CR, Weiner LP (2008) A microfluidic processor for gene expression profiling of single human embryonic stem cells. Lab Chip 8:68–74. https://doi.org/10.1039/b712116d

8. Murphy TW, Zhang Q, Naler LB, Ma S, Lu C (2018) Recent advances in the use of microfluidic technologies for single cell analysis. Analyst 143(1):60–80. https://doi.org/10.1039/c7an01346a

9. Huang Q, Mao S, Khan M, Lin JM (2019) Single-cell assay on microfluidic devices. Analyst 144:808–823. https://doi.org/10.1039/c8an01079j

10. Chen QS, Wu J, Zhuang QC, Lin XX, Zhang J, Lin JM (2013) Microfluidic isolation of highly pure embryonic stem cells using feeder-separated co-culture system. Sci Rep 3:2433. https://doi.org/10.1038/srep02433

11. Mao S, Zhang W, Huang Q, Khan M, Li H, Uchiyama K, Lin JM (2018) In Situ scatheless cell detachment reveals correlation between adhesion strength and viability at single-cell resolution. Angew Chem Int Ed 57(1):236–240

12. Lin L, Lin X, Lin L, Feng Q, Kitamori T, Lin JM, Sun J (2017) Integrated microfluidic platform with multiple functions to probe tumor–endothelial cell interaction. Anal Chem 89(18):10037–10044

13. Rettig JR, Folch A (2005) Large-scale single-cell trapping and imaging using microwell arrays. Anal Chem 77:5628–5634. https://doi.org/10.1021/ac0505977

14. Di Carlo D, Edd JF, Irimia D, Tompkins RG, Toner M (2008) Equilibrium separation and filtration of particles using differential inertial focusing. Anal Chem 80:2204–2211

15. Wang XB, Yang J, Huang Y, Vykoukal J, Becker FF, Gascoyne PR (2000) Cell separation by dielectrophoretic field-flow-fractionation. Anal Chem 72:832–839

16. Evander M, Johansson L, Lilliehorn T, Piskur J, Lindvall M, Johansson S, Almqvist M, Laurell T, Nilsson J (2007) Noninvasive acoustic cell trapping in a microfluidic perfusion system for online bioassays. Anal Chem 79(7):2984–2991. https://doi.org/10.1021/ac061576v

17. Matioli GT, Niewisch HB (1965) Electrophoresis of hemoglobin in single erythrocytes. Science 150(3705):1824–1826

18. Osborne NN, Szczepaniak AC, Neuhoff V (1973) Amines and amino acids in identified neurons of Helix pomatia. Int J Neurosci 5(3):125–131

19. McADOO DJ (1978) The Retzius cell of the leech hirudo medicinalis. In: Osborne NN (ed) Biochemistry of Characterised Neurons. Elsevier, Amsterdam

20. Lent CM, Mueller RL, Haycock DA (1983) Chromatographic and histochemical identification of dopamine within an identified neuron in the leech nervous-system. J Neurochem 41(2):481–490. https://doi.org/10.1111/j.1471-4159.1983.tb04766.x

21. McCaman RE, Weinreich D, Borys H (1973) Endogenous levels of acetylcholine and choline in individual neurons of Aplysia. J Neurochem 21(2):473–476

22. Melamed MR, Lindmo T, Mendelsohn ML, Bigler RD (1991) Flow cytometry and sorting. Am J Clin Oncol 14(1):90

23. Neher E, Sakmann B (1976) Single-channel currents recorded from membrane of denervated frog muscle fibres. Nature 260(5554):799–802

24. Kennedy RT, Stclaire RL, White JG, Jorgenson JW (1987) Chemical-analysis of single neurons by open tubular liquid-chromatography. Mikrochim Acta 2(1–3):37–45

25. Wallingford RA, Ewing AG (1988) Capillary zone electrophoresis with electrochemical detection in 12.7-Mu-M diameter columns. Anal Chem 60(18):1972–1975. https://doi.org/10.1021/ac00169a027

26. Croushore CA, Supharoek SA, Lee CY, Jakmunee J, Sweedler JV (2012) Microfluidic device for the selective chemical stimulation of neurons and characterization of peptide release with mass spectrometry. Anal Chem 84(21):9446–9452. https://doi.org/10.1021/ac302283u

27. Rubakhin SS, Lanni EJ, Sweedler JV (2013) Progress toward single cell metabolomics. Curr Opin Biotechnol 24(1):95–104. https://doi.org/10.1016/j.copbio.2012.10.021

28. Comi TJ, Do TD, Rubakhin SS, Sweedler JV (2017) Categorizing cells on the basis of their chemical profiles: progress in single-cell mass spectrometry. J Am Chem Soc 139:3920–3929. https://doi.org/10.1021/jacs.6b12822

29. Ong TH, Kissick DJ, Jansson ET, Comi TJ, Romanova EV, Rubakhin SS, Sweedler JV (2015) Classification of large cellular populations and discovery of rare cells using single cell matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Anal Chem 87(14):7036–7042. https://doi.org/10.1021/acs.analchem.5b01557

30. Zhang Z, Krylov S, Arriaga EA, Polakowski R, Dovichi NJ (2000) One-dimensional protein analysis of an HT29 human colon adenocarcinoma cell. Anal Chem 72(2):318–322

31. Hu S, Le Z, Newitt R, Aebersold R, Kraly JR, Jones M, Dovichi NJ (2003) Identification of proteins in single-cell capillary electrophoresis fingerprints based on comigration with standard proteins. Anal Chem 75(14):3502–3505

32. Zhu G, Sun L, Yan X, Dovichi NJ (2013) Single-shot proteomics using capillary zone electrophoresis–electrospray ionization-tandem mass spectrometry with production of more than 1 250 *Escherichia coli* peptide identifications in a 50 min separation. Anal Chem 85:2569–2573

33. Qu Y, Sun L, Zhang Z, Dovichi NJ (2018) Site-specific glycan heterogeneity characterization by hydrophilic interaction liquid chromatography solid-phase extraction, reversed-phase liquid chromatography fractionation, and capillary zone electrophoresis-electrospray ionization-tandem mass spectrometry. Anal Chem 90:1223–1233. https://doi.org/10.1021/acs.analchem.7b03912

34. Sibbitts J, Sellens KA, Jia S, Klasner SA, Culbertson CT (2018) Cellular analysis using microfluidics. Anal Chem 90(1):65–85. https://doi.org/10.1021/acs.analchem.7b04519

35. Armbrecht L, Dittrich PS (2017) Recent advances in the analysis of single cells. Anal Chem 89(1):2–21

36. Lin L, Chen QH, Sun JS (2018) Micro/nanofluidics-enabled single-cell biochemical analysis. TrAC-Trend Anal Chem 99:66–74. https://doi.org/10.1016/j.trac.2017.11.017

37. Shear JB, Fishman HA, Allbritton NL, Garigan D, Zare RN, Scheller RH (1995) Single cells as biosensors for chemical separations. Science 267(5194):74–77. https://doi.org/10.1126/science.7809609

38. Huang B, Wu H, Bhaya D, Grossman A, Granier S, Kobilka BK, Zare RN (2007) Counting low-copy number proteins in a single cell. Science 315(5808):81–84. https://doi.org/10.1126/science.1133992

39. Zare RN, Kim S (2010) Microfluidic platforms for single-cell analysis. Annu Rev Biomed Eng 12:187–201. https://doi.org/10.1146/annurev-bioeng-070909-105238

40. Wheeler AR, Throndset WR, Whelan RJ, Leach AM, Zare RN, Liao YH, Farrell K, Manger ID, Daridon A (2003) Microfluidic device for single-cell analysis. Anal Chem 75(14):3581–3586

41. Mellors JS, Jorabchi K, Smith LM, Ramsey JM (2010) Integrated microfluidic device for automated single cell analysis using electrophoretic separation and electrospray ionization mass spectrometry. Anal Chem 82(3):967–973. https://doi.org/10.1021/ac902218y

42. McClain MA, Culbertson CT, Jacobson SC, Allbritton NL, Sims CE, Ramsey JM (2003) Microfluidic devices for the high-throughput chemical analysis of cells. Anal Chem 75(21):5646–5655. https://doi.org/10.1021/ac0346510

43. Broyles BS, Jacobson SC, Ramsey JM (2003) Sample filtration, concentration, and separation integrated on microfluidic devices. Anal Chem 75(11):2761–2767

44. Liu C, Liu J, Gao D, Ding M, Lin J-M (2010) Fabrication of microwell arrays based on two-dimensional ordered polystyrene microspheres for high-throughput single-cell analysis. Anal Chem 82(22):9418–9424

45. Chen Q, Wu J, Zhang Y, Lin Z, Lin J-M (2012) Targeted isolation and analysis of single tumor cells with aptamer-encoded microwell array on microfluidic device. Lab Chip 12(24): 5180–5185

46. Liu J, Gao D, Mao S, Lin J-M (2012) A microfluidic photolithography for controlled encapsulation of single cells inside hydrogel microstructures. Sci China Chem 55(4):494–501

47. Shields CW IV, Reyes CD, López GP (2015) Microfluidic cell sorting: a review of the advances in the separation of cells from debulking to rare cell isolation. Lab Chip 15(5): 1230–1249

48. Thompson AM, Paguirigan AL, Kreutz JE, Radich JP, Chiu DT (2014) Microfluidics for single-cell genetic analysis. Lab Chip 14(17):3135–3142. https://doi.org/10.1039/c4lc00175c

49. Sajeesh P, Sen AK (2014) Particle separation and sorting in microfluidic devices: a review. Microfluid Nanofluid 17(1):1–52. https://doi.org/10.1007/s10404-013-1291-9

50. Chen Y, Li P, Huang PH, Xie Y, Mai JD, Wang L, Nguyen NT, Huang TJ (2014) Rare cell isolation and analysis in microfluidics. Lab Chip 14(4):626–645. https://doi.org/10.1039/c3lc90136j

51. Autebert J, Coudert B, Bidard FC, Pierga JY, Descroix S, Malaquin L, Viovy JL (2012) Microfluidic: an innovative tool for efficient cell sorting. Methods 57(3):297–307. https://doi.org/10.1016/j.ymeth.2012.07.002

52. Mehrishi JN, Bauer J (2002) Electrophoresis of cells and the biological relevance of surface charge. Electrophoresis 23(13):1984–1994. https://doi.org/10.1002/1522-2683(200207)23:13%3c1984:AID-ELPS1984%3e3.0.CO;2-U

53. Voldman J (2006) Electrical forces for microscale cell manipulation. Annu Rev Biomed Eng 8:425–454. https://doi.org/10.1146/annurev.bioeng.8.061505.095739

54. Shim S, Stemke-Hale K, Noshari J, Becker FF, Gascoyne PRC (2013) Dielectrophoresis has broad applicability to marker-free isolation of tumor cells from blood by microfluidic systems. Biomicrofluidics 7(1):011808. https://doi.org/10.1063/1.4774307

55. Burguillos MA, Magnusson C, Nordin M, Lenshof A, Augustsson P, Hansson MJ, Elmer E, Lilja H, Brundin P, Laurell T, Deierborg T (2013) Microchannel acoustophoresis does not impact survival or function of microglia, leukocytes or tumor cells. PLoS ONE 8(5):e64233. https://doi.org/10.1371/journal.pone.0064233

56. Skowronek V, Rambach RW, Schmid L, Haase K, Franke T (2013) Particle deflection in a poly(dimethylsiloxane) microchannel using a propagating surface acoustic wave: size and frequency dependence. Anal Chem 85(20):9955–9959. https://doi.org/10.1021/ac402607p

57. Yeo LY, Friend JR (2014) Surface acoustic wave microfluidics. Annu Rev Fluid Mech 46:379–406. https://doi.org/10.1146/annurev-fluid-010313-141418

58. Ding X, Li P, Lin SC, Stratton ZS, Nama N, Guo F, Slotcavage D, Mao X, Shi J, Costanzo F, Huang TJ (2013) Surface acoustic wave microfluidics. Lab Chip 13(18):3626–3649. https://doi.org/10.1039/c3lc50361e

59. Ashkin A (1970) Acceleration and trapping of particles by radiation pressure. Phys Rev Lett 24(4):156. https://doi.org/10.1103/PhysRevLett.24.156

60. Ashkin A, Dziedzic JM, Bjorkholm JE, Chu S (1986) Observation of a single-beam gradient force optical trap for dielectric particles. Opt Lett 11(5):288

61. Jonas A, Zemanek P (2008) Light at work: the use of optical forces for particle manipulation, sorting, and analysis. Electrophoresis 29(24):4813–4851. https://doi.org/10.1002/elps.200800484

62. Moffitt JR, Chemla YR, Smith SB, Bustamante C (2008) Recent advances in optical tweezers. Annu Rev Biochem 77:205–228. https://doi.org/10.1146/annurev.biochem.77.043007.090225

63. Plouffe BD, Murthy SK, Lewis LH (2015) Fundamentals and application of magnetic particles in cell isolation and enrichment: a review. Rep Prog Phys 78(1):016601. https://doi.org/10.1088/0034-4885/78/1/016601

64. Hejazian M, Li W, Nguyen NT (2015) Lab on a chip for continuous-flow magnetic cell separation. Lab Chip 15(4):959–970. https://doi.org/10.1039/c4lc01422g

65. Zborowski M, Chalmers JJ (2011) Rare cell separation and analysis by magnetic sorting. Anal Chem 83(21):8050–8056. https://doi.org/10.1021/ac200550d

66. Inglis DW, Davis JA, Austin RH, Sturm JC (2006) Critical particle size for fractionation by deterministic lateral displacement. Lab Chip 6(5):655–658. https://doi.org/10.1039/b515371a

67. McGrath J, Jimenez M, Bridle H (2014) Deterministic lateral displacement for particle separation: a review. Lab Chip 14(21):4139–4158. https://doi.org/10.1039/c4lc00939h

68. Di Carlo D (2009) Inertial microfluidics. Lab Chip 9(21):3038–3046. https://doi.org/10.1039/b912547g

69. Martel JM, Toner M (2014) Inertial focusing in microfluidics. Annu Rev Biomed Eng 16:371–396. https://doi.org/10.1146/annurev-bioeng-121813-120704

70. Geislinger TM, Franke T (2014) Hydrodynamic lift of vesicles and red blood cells in flow—from Fåhræus & Lindqvist to microfluidic cell sorting. Adv Colloid Interfac 208:161–176

71. Huang Q, Mao S, Khan M, Zhou Z, Lin J-M (2018) Dean flow assisted-cell ordering system for lipid profiling in single-cells using mass spectrometry. Chem Commun 54:2595–2598

72. Nilsson J, Evander M, Hammarstrom B, Laurell T (2009) Review of cell and particle trapping in microfluidic systems. Anal Chim Acta 649(2):141–157. https://doi.org/10.1016/j.aca.2009.07.017

73. Unger MA, Chou HP, Thorsen T, Scherer A, Quake SR (2000) Monolithic microfabricated valves and pumps by multilayer soft lithography. Science 288(5463):113–116

74. Thorsen T, Maerkl SJ, Quake SR (2002) Microfluidic large-scale integration. Science 298(5593):580–584. https://doi.org/10.1126/science.1076996

75. Kim S-H, Lee GH, Park JY (2013) Microwell fabrication methods and applications for cellular studies. Biomed Eng Lett 3(3):131–137

76. Lindström S, Andersson-Svahn A (2011) Miniaturization of biological assays—overview on microwell devices for single-cell analyses. BBA-Gen Subjects 1810(3):308–316

77. Lutz BR, Chen J, Schwartz DT (2006) Hydrodynamic tweezers: 1. Noncontact trapping of single cells using steady streaming microeddies. Anal Chem 78(15):5429–5435. https://doi.org/10.1021/ac060555y

78. Karimi A, Yazdi S, Ardekani AM (2013) Hydrodynamic mechanisms of cell and particle trapping in microfluidics. Biomicrofluidics 7(2):21501. https://doi.org/10.1063/1.4799787

79. Utada AS, Lorenceau E, Link DR, Kaplan PD, Stone HA, Weitz DA (2005) Monodisperse double emulsions generated from a microcapillary device. Science 308(5721):537–541. https://doi.org/10.1126/science.1109164

80. Tran TM, Lan F, Thompson CS, Abate AR (2013) From tubes to drops: droplet-based microfluidics for ultrahigh-throughput biology. J Phys D Appl Phys 46(11):114004. https://doi.org/10.1088/0022-3727/46/11/114004

81. Guo MT, Rotem A, Heyman JA, Weitz DA (2012) Droplet microfluidics for high-throughput biological assays. Lab Chip 12(12):2146–2155. https://doi.org/10.1039/c2lc21147e

82. Brown RB, Audet J (2008) Current techniques for single-cell lysis. J R Soc Interface 5 Suppl 2(Suppl 2):S131–138. https://doi.org/10.1098/rsif.2008.0009.focus

83. Nan L, Jiang Z, Wei X (2014) Emerging microfluidic devices for cell lysis: a review. Lab Chip 14(6):1060–1073. https://doi.org/10.1039/c3lc51133b

84. Kotlowski R, Martin A, Ablordey A, Chemlal K, Fonteyne PA, Portaels F (2004) One-tube cell lysis and DNA extraction procedure for PCR-based detection of Mycobacterium ulcerans in aquatic insects, molluscs and fish. J Med Microbiol 53(Pt 9):927–933. https://doi.org/10.1099/jmm.0.45593-0

85. Marcus JS, Anderson WF, Quake SR (2006) Microfluidic single-cell mRNA isolation and analysis. Anal Chem 78(9):3084–3089. https://doi.org/10.1021/ac0519460

86. Cichova M, Proksova M, Tothova L, Santha H, Mayer V (2012) On-line cell lysis of bacteria and its spores using a microfluidic biochip. Cent Eur J Biol 7(2):230–240. https://doi.org/10.2478/s11535-012-0005-8

87. Martin-Laurent F, Philippot L, Hallet S, Chaussod R, Germon JC, Soulas G, Catroux G (2001) DNA extraction from soils: old bias for new microbial diversity analysis methods. Appl Environ Microbiol 67(5):2354–2359. https://doi.org/10.1128/AEM.67.5.2354-2359.2001

88. Sad S, Dudani R, Gurnani K, Russell M, van Faassen H, Finlay B, Krishnan L (2008) Pathogen proliferation governs the magnitude but compromises the function of CD8 T cells. J Immunol 180(9):5853–5861

89. Doebler RW, Erwin B, Hickerson A, Irvine B, Woyski D, Nadim A, Sterling JD (2009) Continuous-flow, rapid lysis devices for biodefense nucleic acid diagnostic systems. Jala 14(3):119–125. https://doi.org/10.1016/j.jala.2009.02.010

90. Weaver JC (2000) Electroporation of cells and tissues. IEEE T Plasma Sci 28(1):24–33. https://doi.org/10.1109/27.842820

91. Weaver JC (2003) Electroporation of biological membranes from multicellular to nano scales. IEEE T Dielect El In 10(5):754–768. https://doi.org/10.1109/Tdei.2003.1237325

92. Tsong TY (1991) Electroporation of cell membranes. Biophys J 60(2):297–306. https://doi.org/10.1016/S0006-3495(91)82054-9

93. Hjouj M, Last D, Guez D, Daniels D, Sharabi S, Lavee J, Rubinsky B, Mardor Y (2012) MRI study on reversible and irreversible electroporation induced blood brain barrier disruption. PLoS ONE 7(8):e42817. https://doi.org/10.1371/journal.pone.0042817

94. Fox MB, Esveld DC, Valero A, Luttge R, Mastwijk HC, Bartels PV, van den Berg A, Boom RM (2006) Electroporation of cells in microfluidic devices: a review. Anal Bioanal Chem 385(3):474–485. https://doi.org/10.1007/s00216-006-0327-3

95. Wang S, Lee LJ (2013) Micro-/nanofluidics based cell electroporation. Biomicrofluidics 7(1):11301. https://doi.org/10.1063/1.4774071

96. Vogel A, Busch S, Jungnickel K, Birngruber R (1994) Mechanisms of intraocular photodisruption with picosecond and nanosecond laser pulses. Lasers Surg Med 15(1):32–43

97. Shaw S, Jin Y, Schiffers W, Emmony D (1996) The interaction of a single laser-generated cavity in water with a solid surface. J Acoust Soc Am 99(5):2811–2824

98. Vogel A, Noack J, Nahen K, Theisen D, Busch S, Parlitz U, Hammer DX, Noojin GD, Rockwell BA, Birngruber R (1999) Energy balance of optical breakdown in water at nanosecond to femtosecond time scales. Appl Phys B-Lasers O 68(2):271–280. https://doi.org/10.1007/s003400050617

99. Sims CE, Meredith GD, Krasieva TB, Berns MW, Tromberg BJ, Allbritton NL (1998) Laser-micropipet combination for single-cell analysis. Anal Chem 70(21):4570–4577

100. Dhawan MD, Wise F, Baeumner AJ (2002) Development of a laser-induced cell lysis system. Anal Bioanal Chem 374(3):421–426. https://doi.org/10.1007/s00216-002-1489-2

101. Quinto-Su PA, Lai HH, Yoon HH, Sims CE, Allbritton NL, Venugopalan V (2008) Examination of laser microbeam cell lysis in a PDMS microfluidic channel using time-resolved imaging. Lab Chip 8(3):408–414. https://doi.org/10.1039/b715708h

102. Cordero N, West J, Berney H (2003) Thermal modelling of Ohmic heating microreactors. Microelectron J 34(12):1137–1142. https://doi.org/10.1016/S0026-2692(03)00204-0

103. Fu R, Xu B, Li D (2006) Study of the temperature field in microchannels of a PDMS chip with embedded local heater using temperature-dependent fluorescent dye. Int J Therm Sci 45(9):841–847. https://doi.org/10.1016/j.ijthermalsci.2005.11.009

104. Waters LC, Jacobson SC, Kroutchinina N, Khandurina J, Foote RS, Ramsey JM (1998) Microchip device for cell lysis, multiplex PCR amplification, and electrophoretic sizing. Anal Chem 70(1):158–162

105. Zhu K, Jin H, Ma Y, Ren Z, Xiao C, He Z, Zhang F, Zhu Q, Wang B (2005) A continuous thermal lysis procedure for the large-scale preparation of plasmid DNA. J Biotechnol 118(3):257–264. https://doi.org/10.1016/j.jbiotec.2005.05.003

106. Junttila MR, de Sauvage FJ (2013) Influence of tumour micro-environment heterogeneity on therapeutic response. Nature 501(7467):346–354. https://doi.org/10.1038/nature12626
107. Junker JP, Noel ES, Guryev V, Peterson KA, Shah G, Huisken J, McMahon AP, Berezikov E, Bakkers J, van Oudenaarden A (2014) Genome-wide RNA Tomography in the zebrafish embryo. Cell 159(3):662–675. https://doi.org/10.1016/j.cell.2014.09.038
108. Chen KH, Boettiger AN, Moffitt JR, Wang S, Zhuang X (2015) RNA imaging. Spatially resolved, highly multiplexed RNA profiling in single cells. Science 348(6233):aaa6090. https://doi.org/10.1126/science.aaa6090