Microfluidic Cell Sorting and Separation Technology

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Abstract Cell sorting and separation is widely used as a critical first step for research and clinical applications where it is needed to isolate individual cell types from a heterogeneous biological sample. In this introductory chapter, we review conventional cell sorting and separation techniques and their applications. To meet the complex and diversifying needs for cell sorting, many microfluidic techniques based on diverse sorting criteria have been developed recently. Microfluidics has many advantages including variety of sorting principles, precise cell manipulation capability, and combination with downstream analysis. We highlight microfluidic cell sorting and separation techniques and their principles, and establish terminologies and metrics used in their analysis. Lastly, we provide perspective of potential future applications or directions for microtechnologies.

Keywords Cell sorting · Cell separation · Microfluidics · Magnetophoresis · Dielectrophoresis · Optical sorting · Acoustic sorting · Gravity-driven cell manipulation · Inertial microfluidics · Aqueous two-phase system · FACS · MACS · Centrifugation · Filtration · Terminology

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1 Introduction

Manipulation and sorting of biological cells has seen ever increasing widespread use in medicine, biotechnology, and cellular biology. Extracted biofluids are often heterogenous in composition, and depending on their source of origin can possess a mixture of cell types (white blood cells, red blood cells, circulating progenitor cells, malignant cells), and biomolecules (plasma, proteins, antibodies). Cell manipulation and sorting are often a critical first step to either separate samples into constituent cell populations/components, or to isolate a desired cell type from a complex biofluid. Traditionally, this task is accomplished with fluorescent-activated cell sorting (FACS), or magnetic-activated cell sorting (MACS). However, these traditional methods are hampered by several limitations including large, unwieldy instrumentation, low sample throughput, cell death, limited quantitation capability, or high costs.

Limitations of existing traditional techniques, alongside the advent of personalized medicine (either for personalized diagnostics or developing patient-specific cell therapies/treatments) has generated tremendous need for modernized devices and systems that either possess reduced costs, higher throughput, improved specificity, or portability.

Microtechnologies/microdevices are looked toward as the solution to these issues. Operating at scales similar to biological structures, these devices possess inherent scalability and low cost due to microfabrication techniques, inherent portability due to operating at the size limit of biology, while potentially possessing higher throughputs due to parallelized designs or unique parallel physical manipulation methodologies.

In this introductory chapter, we will highlight conventional cell sorting and separation techniques (including label-free and antibody-based approaches) and their applications, microfluidic techniques and principles (and establish terminologies and metrics used in their analysis), and provide perspective of potential future applications or directions for microtechnologies in biological sample handling.

2 Conventional Cell Sorting and Separation Techniques

Biological samples, such as blood, bone marrow, and tissues consist of different types and lineages of cells. As a result, studies with such heterogeneous samples require a sample preparation step that can yield a purified cell population to avoid biased or erroneous results. Conventional cell separation and sorting techniques allow classification and separation of cells based on characteristics of cells including size, density, and cell contents, such as proteins and DNA (Orfao and Ruiz-Argüelles 1996; Almeida et al. 2014). Advances in monoclonal antibodies led to a dramatic increase in the use of immunologic methods to identify cell contents, while label-free methods such as centrifugation and filtration are also widely used as a preparatory step for analysis or further sorting and separation. Here summarized are frequently used conventional cell separation and sorting techniques (Table 1).

Table 1 Comparison of conventional cell sorting techniques

	Technique	Principle	Pros	Cons
Label-free	Filter	Size	Simple process Low cost High-throughput	Low purity Low yield
	Centrifugation	Density		
	Adherence	Adherence		
	Culture	Growth		
Antibody-based	Panning	Antibody	High purity High yield	Complexity of labeling High cost Labeling may change cell function
	FACS	Antibody		
	MACS	Antibody		

2.1 Label-Free Techniques

Label-free cell separation techniques separate cells based on physical properties of cells, such as size, deformability, electrical polarizability, adhesion, and density. Widely used label-free techniques include filtration, centrifugation (and sedimentation), cell adherence-based separation, and cell culture. These techniques allow the separation of large numbers of desired cells in relatively simple ways. More importantly, cells separated using label-free techniques are readily available for subsequent analysis and even for therapeutic purposes. However, the separation is achieved in a qualitative way rather than a quantitative way and the separation purity is generally low.

Cell separation by *filtration* is a simple and inexpensive method to separate cells by size and/or deformability using filters with uniform microscale meshes or pores. Filtration is typically used as a pre-enrichment step for further cell purification, and it is especially useful in preparing single cell suspensions by removal of cell aggregates and large particles. The cell separation filters traditionally are made of cotton wool columns or copper filters, and recently polymer meshes, for example, made with nylon and polyethylene terephthalate (PET) are replacing them. A notable disadvantage of filtration is the significant amount of cell loss during the process. Filtration is also used to concentrate and retain larger cells for sample preparation prior to cytological analysis (e.g., ThinPrep®).

Centrifugation (or *sedimentation*) separates cells by their differences in density. Centrifugation is an extensively used cell separation technique because it is suitable for separation of large numbers of cells in a relatively simple and inexpensive way. Although it is not as significant as in filtration, centrifugation also has problems associated with low purity and loss of target cells. The low purity can be overcome by repeated centrifugations using different conditions (density of medium, and angular velocity). Alternatively, density gradient centrifugation can provide more efficient and practical cell separation results (Carmignac 2002). For this purpose, Percoll or other gradient media (e.g., polysaccharides, iodinated gradient media) are prepared to create isopycnic density gradients. Cells with different densities settle to their isopycnic points via centrifugation. Rosetting is also

a widely used separation technique based on density to deplete a cell population, which is a combination of antibody binding and centrifugation (Strelkauskas et al. 1975; Slaper-Cortenbach et al. 1999). Antibody-enabled binding with a linker between nontarget cells and erythrocytes leads to formation of aggregates or immuno-rosettes, which are denser than the other cell types of interest cells and can be removed by centrifugation.

Cell adherence to a substrate and cell culture can also be used as separation techniques. Cell adherence-based separation enriches desired cells by removing cells that do not attach onto a cell substrate. The method relies on a cell's adhesion capacity (without specific target binding), which is often shared by many different cell types. Therefore, adherence-based separation is used only when high purity is not required or depletion of a specific cell type is needed. The separation is performed typically on a tissue culture plastic dish but more refined methods use glass beads grafted with polymer brushes (Nagase et al. 2012) or micro/nanostructured surfaces (Didar and Tabrizian 2010). An important cell type that was first isolated using such an approach from bone marrow aspirates are mesenchymal stem cells, also called marrow stromal cells (MSCs). These cells spread and adhere strongly to these rigid plastic surfaces.

Cell culture using media that stimulates or inhibits the growth of certain cell types can be used as a cell separation technique. For example in the process of bone marrow transplants, long-term bone marrow culture in controlled media can be used to selectively expand hematopoietic stem cells (Devine et al. 2003). These methods can provide a relatively homogeneous cell population; however, the resulting sample is not the original cells but expanded cells.

2.2 Techniques Based on Antibody Binding

High purity cell separation and sorting can be achieved by the use of a monoclonal antibody that binds to a cellular component. Typically, an antibody is selected to identify a single (or a few) cell surface markers and the antibody is conjugated with fluorescent molecules or attached to microparticles to separate target cells. Owing to high specificity of antibody—antigen binding, antibody-based separation and sorting techniques can provide much higher purity compared to label-free techniques. However, antibody-based techniques have some disadvantages related with labeling. First, labeling with fluorescent molecules and antibodies may affect cell fate and functions, which affects downstream analysis and efficacy of therapeutics. Second, a labeling process is often time-consuming and labor-intensive, which adds difficulty and cost. Lastly, for a practical separation and sorting application the choice of antibodies is limited within a pool of commercially available antibodies, which in turn limits the separation targets to those cells with specific markers. Widely used antibody-based cell separation techniques include cell panning, MACS, and FACS.

With the *cell panning* technique, cells having specific antigens can be selectively attached on an antibody-coated surface. Typically, antibodies are adsorbed to a plastic surface, such as petri dishes or polymer microparticles. Cell panning can provide high purity but high cell loss is unavoidable, and quantitative separation based on surface expression is not achievable, yielding only a binary separation. Compared to other antibody-based techniques, it is easier to release cells and the separated cells can be used for further analysis or therapeutics.

MACS employs antibody-conjugated magnetic beads to target the specific marker on the cell surface. Cells labeled with magnetic beads can be selectively collected under a magnetic field produced by a permanent magnet. MACS is hugely benefited by the well-established technology for magnetic particles. Magnetic particles are commercially available with diversity not only in size and material but also with surface modification or antibody conjugation. MACS allows significantly higher throughput but lower purity then FACS, because cells with a few bound magnetic particles compared to many particles are both concentrated in the magnetic field. That is, like panning, the separation cannot quantify the amount of surface antigen on a cell. Another notable limitation is the difficulty of detachment and removal of the beads after separation.

FACS is one of the most powerful cell sorting techniques that is based on flow cytometry. A flow cytometer allows the analysis and classification of individual cells by multiparameter optical measurements. Cells are hydrodynamically focused to a narrow stream and pass optical interrogation points one by one where laser beams illuminate individual cells. At this point scattered light and fluorescent signals are generated and detected by multiple detectors. Sorting decisions are made based on these signals, which provide quantitative information on the size of the cells and the amount of the fluorescent-labeled antibodies bound to cell membrane and/or internal structures of the cells. Modern flow cytometers can offer throughput in the range of 10,000 cells/s, which is fairly high but lower than bulk sorting techniques like MACS or centrifugation. To sort the individual cells, the cell stream is ejected into air and broken up into droplets containing no more than one cell per droplet. The droplet formation can be influenced by many parameters including orifice size and temperature. The droplets are electrically charged depending on the sort decision and then the droplets are diverted into separate containers based on their charge by using an electrostatic deflection system. While FACS provides high purity quantitative sorting decisions throughput is not sufficient for sorting of therapeutic cells, and cells are often damaged during the sorting process. Limited throughput also prevents FACS from use in certain applications such as rare cell sorting. High equipment cost (typically >$100,000), in addition to high operation and material costs, is one other notable limitation. The process of droplet formation also produces aerosols, which is a potential biohazard to a user and appropriate safety measures should be taken.

2.3 Applications

By enabling the study of individual cell types isolated from a heterogeneous population, cell separation, and sorting is widely used for research in cell biology and many other fields including molecular genetics and proteomics (Orfao and RuizArguelles 1996; Mattanovich and Borth 2006; Gossett et al. 2010; Autebert et al. 2012; Almeida et al. 2014; Shields et al. 2015). Especially, in the field of cancer, stem cells and immunology rare cell separation and sorting receives increasing attention. In clinical fields, preparation of homogeneous, purified cell populations is essential for immunology, oncology, hematology, tissue engineering, and regenerative medicine.

Cell sorting and separation has been extensively used for blood because it is a necessary step not only to collect cell-free plasma, but also to sort the different types of RBCs and WBCs. Blood is extremely rich in information about the physiological state of the body, which can be extracted from the genetic material, protein disease markers, and cellular components within blood. Especially, blood cells, which represent ~45 % of blood volume (~10^9 RBCs and ~10^6 WBCs in 1 mL blood), are often used for hematological tests, diagnosis of disease, gene expression profiling, and therapeutics. Despite the importance, the separation of pure cell populations from blood is still a challenging task due to blood cell diversity and susceptibility to alteration during the handling procedures. Centrifugation and FACS or MACS have been generally used for blood separation but recent studies suggest lab-on-a-chip microscale or microfluidic approaches can address many challenges (Toner and Irimia 2005).

Cell-based therapy is one of the fields that can be most benefited from advanced cell sorting techniques because infusion of high purity cells can increase therapeutic efficacy. In case of bone marrow transplants, patients have received transplants of whole human leukocyte antigen (HLA)-identical bone marrow to avoid the risk of graft versus host disease with the finding of hematopoietic stem cells, transplantation of purified CD34+ cells from bone marrow has been performed (Beaujean 1997). Recently, peripheral stem cell transplantation of MSCs has also became a more common procedure due to its less invasive nature (Handgretinger et al. 1998; Despres et al. 2000), and regulatory approval for use in treatment of a variety of diseases. More recently, cell immunotherapies, including engineered T-cell receptor and chimeric antigen receptor T-cells have shown significant promise in programming one's own immune system to attack cancer. In the normal process of cell isolation and further upon transduction with engineered receptors and expanding cell clones, separation approaches are used.

As can be seen from an example of bone marrow transplantation, while traditional applications of cell sorting focused on the enrichment of larger populations of desired cells, recent focus has expanded to sorting of rare cells, which include circulating tumor cells (CTCs), circulating endothelial cells (CECs), and endothelial progenitor cells (EPCs), stem cells, fetal cells, infected cells, and bacteria.

CTCs, for example, are related to cancer metastasis and can be found in blood at very low abundance (1–100 cells/mL). Not only are CTCs extremely rare compared to a large population of RBCs and WBCs, their heterogeneity complicates the sorting; antibody-based sorting has relied on binding to epithelial markers (EpCAM), however, tumor cells can undergo epithelial–mesenchymal transition and may not express EpCAM (Thiery 2002; Kalluri and Weinberg 2009). Instead of antibodies, physical properties such as size and deformability could be used for CTC enrichment (Cima et al. 2013; Jin et al. 2014; Low and Abas 2015). Rare cell separation requires high-throughput while maintaining high purity and yield (low loss). MACS provides very high-throughput but it does not allow labeling based on multiple markers and detachment of collected cells is difficult. FACS can analyze multiple signals yet its throughput is still a limiting factor for rare cells.

3 Microfluidic Cell Sorting Technology

Advances in genomics and cell biology have significantly increased the complexity of sorting criteria and previously known-to-be homogeneous cell populations may be further classified into different subgroups using new sorting criteria. As a result, conventional techniques based on a few sorting principles would be insufficient to deliver proper sorting strategies. On the other hand, microfluidic technology is expected to provide better solutions with its unique advantages (Pamme 2007; Chen et al. 2008; Didar and Tabrizian 2010; Gossett et al. 2010; Lenshof and Laurell 2010; Autebert et al. 2012; Gao et al. 2013; Sajeesh and Sen 2014; Shields et al. 2015). Several noteworthy advantages of microfluidics for cell sorting and separation applications are: (1) The laminar nature of fluid flow at these scales allows confinement of cells within a narrow controlled stream line. (2) The flow field can have velocity gradients over the scale of cells which allows separation mechanisms that are not possible in the macroscale (e.g., hydrodynamic separations). (3) Small device dimensions allows the generation of strong electric or magnetic fields and their gradient. (4) High surface to volume ratio increase the chance of surface binding of cells. (5) Multiple microfluidic devices can be integrated to perform separation and downstream analysis of cells seamlessly. In addition, general advantages of microfluidic techniques apply, such as easy multiplexing for higher throughput, rapid, and low cost operation, and reduced requirements for a skilled user with automation.

3.1 Terminology for Cell Manipulation

Microfluidic technology enables diverse cell handling techniques, which include physical and biochemical analysis, cell patterning, cell culture, and cell manipulation. Among these, the focus of this book is on cell manipulation techniques,

especially on cell sorting and separation. Cell manipulation refers to general operations that involve physical methods to control a cell's position, orientation, or shape. Cell sorting conventionally refers to a procedure that can separate, isolate, or enrich a specific cell type. The usage of the term cell separation has increased with development of diverse microfluidic-based cell sorting techniques. Although sorting and separation have been often used with the same meaning, it is more common to use them in different situations within the microfluidics community. Cell sorting can be defined as a process to separate cells based on their properties. In the sorting process, for each of the cells, an identification is made and a decision is followed whether to sort or not. For example, FACS can identify cells by optical properties and following this a sorting action is performed according to the predetermined sorting gates. In contrast to cell sorting, cell separation usually refers to a process that does not involve a sort decision, that is, a passive process. In a separation process, a physical property of the cell itself serves as the basis for a cell being accumulated or not. For example, filtration of cells involve the process that cells larger than a pore size get trapped, where identification of cell size that is trapping take places at the same time at the filter. Since there is no individual active decision-making process, one cannot choose cells to sort.

3.2 Performance Metrics

Different sorting and separation techniques have different capabilities, which may lead to trade-offs in performance for varying applications. Here, we summarize definitions of these performance metrics to help in comparing different techniques and the trade-offs between performance throughout the many techniques described in the following chapters.

Purity is the **ratio between the number of target cells and the number of total sorted cells**. In case the sorted cell population contains unwanted cells, purity will have a low value. When a sorting technique reports high purity near 100 %, collected cells may be still mixed with unwanted cell populations if the characteristic that is used as the basis of the sorting decision or separation is shared by multiple types of cells. Therefore, a value of purity may vary significantly depending on how one defines "target cells." Without knowing the exact definition of target cells and samples used, it would be difficult to make a fair comparison between different techniques.

Yield or *recovery* is the **ratio between the number of target cells collected and the number of target cells in the original sample**. Yield usually is less than unity not only because sorting capabilities are not perfect but also target cells in the original sample may be lost by lysis, adhesion to device surfaces, and retention within the device or tubing. Yield can be an especially important parameter in case of rare cell sorting.

Enrichment or *enrichment ratio* can be defined as the **ratio between the number of target cells at the inlet divided by total cells and the number of target**

cells at the outlet divided by total cells. Enrichment can be larger than 1 for enrichment and smaller than 1 for depletion. While 'yield' focuses on the description of loss of target cells, 'enrichment' focuses on concentration of the specific cells compared to the other cells. Therefore, *enrichment ratio* is often used to emphasize the separation capability.

Efficiency can be defined as the **ratio between the number of sorted target cells and the number of target cells identified to sort**. Efficiency can be defined for cell sorting where sorting and identification are separate processes. In case of cell separation, efficiency is often used to indicate the same meaning as yield.

Throughput of cell separation is typically expressed as **the number of processed cells per second**. The unit of throughput can vary depending on the application. For a continuous separation technique, a volumetric flow rate and a cell concentration can be given. There is typically trade-offs between throughput and other performance. For example, high cell concentration can cause errors in sorting decisions, which leads to lower purity and yield. For microfluidic devices, throughput per foot print (device area) may provide basis for fair comparisons, because throughput of many microfluidic devices can be easily multiplied by use of parallel channels.

3.3 Principles of Microfluidic Cell Manipulation

When a cell suspension is first introduced into a microfluidic channel, the position of the cells in the cross-section are intrinsically random. The goal of cell manipulation using microfluidics is mainly to control the cell positions relative to the fluid or other cells through a variety of means: Cells can be trapped, focused, moved into different solutions (washing and solution exchange), separated, and sorted (enrichment). Other manipulation operations can include deformation (shape change such as stretching), mechanical lysis, and rotation. To achieve cell manipulation, one needs to apply a force on cells against the drag force from the surrounding fluid that is either stagnant or flowing. The methods that generate forces on cells can be categorized into three groups: (1) use of direct contact with device structures, (2) use of force fields such as gravity, electric fields, or magnetic fields, and (3) use of hydrodynamic drag and lift forces. Microstructures such as filters, or pillars can exert direct mechanical force onto cells by contact, which allows trapping of cells or shifting cells to different streamlines. The use of external force fields such as electromagnetic fields can also provide efficient methods to manipulate cells. For example, an optical tweezer has long been used to trap and move suspended cells and gravitational force acting on cells results in sedimentation of cells. Another important method to manipulate cells in microfluidic systems is to use the secondary flows that are flowing orthogonal to the main fluid flow along the channel. Combined with the laminar nature of microfluidic flows, hydrodynamic methods are very efficient cell manipulation methods in microfluidic systems because the viscous drag becomes significantly larger than other forces at

small scales. Fluid flows can exert a drag force along the flow direction and a lift force orthogonal to the flow direction. Inertial lift forces can be used to manipulate cells at finite Reynolds number flow conditions ($Re > 1$), where Reynolds number (Re) describes the ratio of inertial to viscous forces in the flow.

Similar to conventional sorting techniques, microfluidic sorting techniques also can be categorized into label-free techniques and antibody-binding-based techniques. Because the use of antibodies provides high purity separation and important sorting criteria, there have been many studies of microfluidic-based cell sorting and separation involving antibodies (Du et al. 2007; Nagrath et al. 2007; Didar and Tabrizian 2010). Microfluidic systems combined with optical sensing or imaging capabilities can sort fluorescent-labeled cells by switching flow paths to multiple outlets (Kruger et al. 2002; Johansson et al. 2009). Unlike FACS, microfluidic cell sorting devices are closed systems and contamination and safety issues are less of a concern. Antibody-coated micro or nano particles are also widely used (Inglis et al. 2004; Xia et al. 2006; Adams et al. 2008). Diverse microfluidic techniques are utilized to guide cells bound with the particles. Alternatively cells can be collected in microchannels with immobilized antibodies (Nagrath et al. 2007). In this case the large surface-area-to-volume ratio of microfluidic structures can significantly enhance the capture efficiency.

Microfluidic separation techniques are mostly focused on label-free techniques, by which one can utilize a variety of physical principles to manipulate cells. Label-free techniques can be grouped into (1) *passive manipulation* and (2) *active manipulation* depending on their physical principles (Table 2). Passive manipulation techniques uses microfluidic devices predesigned to perform a specific manipulation operation. For example, a deterministic lateral displacement (DLD) device separates cells based on their size; with cell-to-wall interactions and the laminar nature of flow, cells larger, and smaller than a critical diameter follow a different

Table 2 Comparison of microfluidic cell separation and sorting techniques

	Separation technique	Mode of separation	Separation criteria
Passive	Mechanical Filter	Size exclusion	Size, deformability
	Hydrodynamic	Streamline manipulation	Size, shape
	DLD	Migration in micropost array	Size, shape
	Microstructure	Microstructure perturbation of cell flow	Size, density, deformability
	ATPS	Differential affinity	Surface property and net charge
	Inertial	Lift force and secondary flow	Size, shape, deformability
Active	Electric	Dielectrophoresis	Polarizability, size
	Magnetic	Differential magnetic mobility	Intrinsic magnetic susceptibility
	Acoustic	Acoustic radiation force	Size, density, compressibility
	Optical	Optical lattice	Refractive index, size
	Gravity	Sedimentation difference	Size, density

flow stream line. As the name suggests the particle motion is deterministic, and the operation is *passive*. On the other hand, active manipulation involves a force field that can be *actively* controlled by the operator. For example, cells with different polarizability experience different forces within a nonuniform AC electric field. Using this property cells can be separated by dielectrophoresis (DEP).

4 Future Directions and Outlook

As you will see throughout the following chapters, microfluidic systems and microscale devices using a variety of active and passive approaches have been shown to have unique advantages for cell manipulation, sorting, and separation. Future work must now apply these techniques to applications with significant unmet needs, integrate with analysis approaches to achieve clinically actionable information, and scale through parallelization to throughputs of importance in developing cell therapies. Some of these activities are now well-suited for industry to tackle, and along these lines, we see increasing investment into companies focused on cell sorting and manipulation, which will in the end lead to the translation and use of these technologies.

One future direction is in connecting advantages of microscale manipulation to suitable research or clinical needs. In the research setting, it is becoming clear that populations of cells once thought to be homogenous have significant heterogeneity. Using techniques such as single cell RNA-sequencing of individual cells, or mass flow cytometry (CyTOF) in the last few years has led to uncovering of a range of subpopulations of cells, however, these processes are destructive to cells. One goal would be to develop microscale tools to probe a variety of molecular or phenotypic markers in parallel to better classify cells in a method compatible with downstream separation. One could imagine multiparameter panning or sampling and analysis of small amounts of intracellular components in a nondestructive manner. Drop-based compartmentalization may also allow sampling after disruption of cell membranes for significant periods of time without cell death, because cellular components remain at relatively high concentration in the droplet until the membrane seals. Sorting and separation of nonmammalian cells is also becoming an important area, whether for studying algae that produce biofuels or concentrating bacteria to identify blood stream infections. Sorting is in essence a process of selection, and we anticipate continuous sorting or separation systems combined with culture and mutagenesis can be developed in the future to select and evolve cellular traits of interest for scientific research and clinical production of cell therapies.

Integration of separation with downstream analyses is also an important direction to yield complete clinical solutions. For example, for CTCs, which can provide information about a patient's tumors to direct therapy, separation alone does not provide information, but requires downstream analysis, e.g., enumeration, or measurement of mutations in the genome to provide clinically actionable

information. Techniques that can combine separation seamlessly with downstream workflows should be enabled by microfluidic systems and will be extremely valuable in the future.

Throughput for most single-device microfluidic systems is relatively low, which is not compatible with separations that are needed for emerging cell therapy technologies where tens of millions to billions of cells should be purified. This is particularly difficult for technologies that rely on cell surface antigens, which are most likely to be relevant for cell therapy-based purification. New ways of parallelizing active sorting decisions or developing more quantitative MACS-type approaches which already possess high-throughput should be investigated.

Because of the many exciting developments, microscale cell manipulation technologies have garnered significant commercial investment. This is helping bring research-grade proof-of-concepts to real products that are being used and developed for a range of assays. A range of new companies are being well funded in this space, such as Berkeley Lights, which is commercializing optoelectronic tweezer technology, as well as a number of companies focused on the problem of isolating CTCs, including Vortex Biosciences, which is developing vortex trapping microfluidic cartridges for CTC isolation, Clearbridge Biomedics, commercializing inertial microfluidic-based CTC separation chips, and ApoCell, developing a DEP-based enrichment technique. Commercial successes may then drive future investment into this field, especially targeting the important problem of reducing the cost of cell therapies—from stem cell to immunotherapies. Indeed, the future in cell separation is looking *small*!

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