REVIEW

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The present and future role of microfluidics in biomedical research

Eric K. Sackmann[1], Anna L. Fulton[2] & David J. Beebe[3]

Microfluidics, a technology characterized by the engineered manipulation of fluids at the submillimetre scale, has shown considerable promise for improving diagnostics and biology research. Certain properties of microfluidic technologies, such as rapid sample processing and the precise control of fluids in an assay, have made them attractive candidates to replace traditional experimental approaches. Here we analyse the progress made by lab-on-a-chip microtechnologies in recent years, and discuss the clinical and research areas in which they have made the greatest impact. We also suggest directions that biologists, engineers and clinicians can take to help this technology live up to its potential.

More than a decade ago, we wrote that "microfluidics has the potential to significantly change the way modern biology is performed"[1]. Indeed, we were part of a chorus of researchers that recognised the possibility of new microfluidic tools making substantial contributions to biology and medical research[2–5]. The optimism surrounding microfluidics was well warranted, given the compelling advantages that microfluidic approaches could possibly have over traditional assays used in cell biology. Conceptually, the idea of microfluidics is that fluids can be precisely manipulated using a microscale device built with technologies first developed by the semiconductor industry and later expanded by the micro-electromechanical systems (MEMS) field. These devices, commonly referred to as miniaturized total analysis systems (μTASs)[6,7] or lab-on-a-chip (LoC) technologies, could be applied to biology research to streamline complex assay protocols; to reduce the sample volume substantially; to reduce the cost of reagents and maximize information gleaned from precious samples; to provide gains in scalability for screening applications and batch sample processing analogous to multi-well plates; and to provide the investigator with substantially more control and predictability of the spatio-temporal dynamics of the cell microenvironment.

The field of microfluidics is characterized by the study and manipulation of fluids at the submillimetre length scale. The fluid phenomena that dominate liquids at this length scale are measurably different from those that dominate at the macroscale (Box 1). For example, the relative effect of the force produced by gravity at microscale dimensions is greatly reduced compared to its dominance at the macroscale. Conversely, surface tension and capillary forces are more dominant at the microscale; these forces can be used for a variety of tasks, such as passively pumping fluids in microchannels[8]; precisely patterning surfaces with user-defined substrates[9]; filtering various analytes[10]; and forming monodisperse droplets[11] in multiphase fluid streams for a variety of applications. These examples represent only a fraction of the myriad problems that microfluidic technologies have attempted to address.

The development of comprehensive microfluidic solutions to address problems in biology and clinical research has been embraced by engineers. However, despite material advances in microfluidics as a technology platform, the adoption of novel μTAS techniques in mainstream biology research has not matched the initial enthusiasm surrounding the field[12]. Some argue the technology is still in search of a 'killer application', where the sample-to-answer concept provides a solution that greatly outperforms current methods[13,14]. In this perspective, we will examine the impact of microfluidic technologies on cell biology and medical research within the past decade. We discuss some of the barriers to adoption of microfluidic technologies in mainstream biomedical research, and use a case study to illustrate and highlight these challenges. We focus our attention on recent developments in the field that are facilitating the application of microfluidic technologies to solving problems in diagnostics and biology research. In this area, we highlight the innovative use of different materials that are more optimally suited to performing a given task; and we examine how researchers are taking advantage of μTAS methods to enable scientific inquiry in ways that were not possible using traditional methods. Finally, we will discuss positive trends in the field and infer lessons that can be applied to future microfluidic technology development.

The impact of microfluidics on biomedical research

A primary goal for much of the microfluidics community is to develop technologies that enhance the capabilities of investigators in biology and medical research. Many microfluidic studies describe methods that aim to replace traditional macroscale assays, and usually perform proof-of-concept (PoC) experiments that attempt to demonstrate the efficacy of the new approach. These novel microfluidic methods are usually published in journals that might be characterized as 'engineering' journals, or publications whose readership comprises largely engineers and other members of the physical sciences (for example, chemists and physicists). If publishing PoC studies in engineering journals represents the development phase for a novel biology assay, then the implementation of the technique can be characterized as when the technology is used and published in a biology or medical journal. After all, the stated goal of virtually all PoC studies is to demonstrate new technologies that enable biologists in their everyday research.

We measured the use of microfluidic technologies in mainstream biomedical research over the past decade to assess their impact beyond the engineering community (Fig. 1). In order to identify broad trends of what journals have published papers that use microfluidics (search terms "microfluidic*" and "nanofluidic*"; see Fig. 1 legend), we defined three categories: (1) 'engineering' journals (for example, *Lab on a Chip*, *Small*, *Analytical Chemistry*); (2) 'biology and medicine' journals (for example, *Blood*, *Cell*, *Journal of Clinical Investigation*); and (3) 'multidisciplinary' journals (for example, *Nature*, *Science*, *Proceedings of the National Academy*

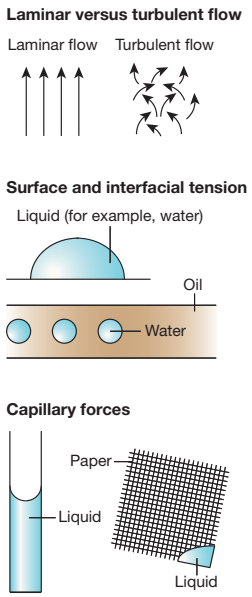
[1]Materials Science Program, Department of Biomedical Engineering, Wisconsin Institutes for Medical Research, University of Wisconsin-Madison, 1111 Highland Avenue, Madison, Wisconsin 53705-2275, USA. [2]Wendt Commons Library, University of Wisconsin-Madison, 215 North Randall Avenue, Madison, Wisconsin 53706, USA. [3]Department of Biomedical Engineering, Wisconsin Institutes for Medical Research, University of Wisconsin-Madison, 1111 Highland Avenue, Room 6009, Madison, Wisconsin 53705-2275, USA.

BOX 1
Useful microfluidics concepts

Laminar versus turbulent flow. The Reynolds number (Re) is a dimensionless quantity that describes the ratio of inertial to viscous forces in a fluid. Re is proportional to the characteristic velocity of the fluid and the length scale of the system; it is inversely proportional to the fluid viscosity. High-Re (~2,000) fluids have flow profiles that increasingly mix stochastically (turbulent flow; Box 1 Figure below). For microfluidic systems, Re is almost always in the laminar flow regime, allowing for highly predictable fluid dynamics. Molecular transport also changes dramatically at this scale because convective mixing does not occur, enabling predictable diffusion kinetics.

Surface and interfacial tension. Surface tension describes the tendency of a fluid in a surface to reduce its free energy by contracting at the surface–air interface. Interfacial tension is a similar phenomenon, but is generally applied to two immiscible fluids (for example, oil and water). These forces play more dominant roles on the microscale (Box 1 Figure below) compared to gravity, which is much more dominant on the macroscale. Researchers have used these phenomena to conduct protein and cell sorting, perform nanoreactions for protein crystallization, and passively drive fluids through microchannels.

Capillary forces. Capillary action describes the movement of a fluid through a narrow constriction, such as a narrow tube or porous material (Box 1 Figure below). At the microscale, capillary action is a more dominant force, allowing fluids to advance in opposition to gravity. Capillary forces have been used to manipulate fluids in many applications, the most famous examples perhaps being the at-home pregnancy test and portable glucometers to monitor blood glucose levels.



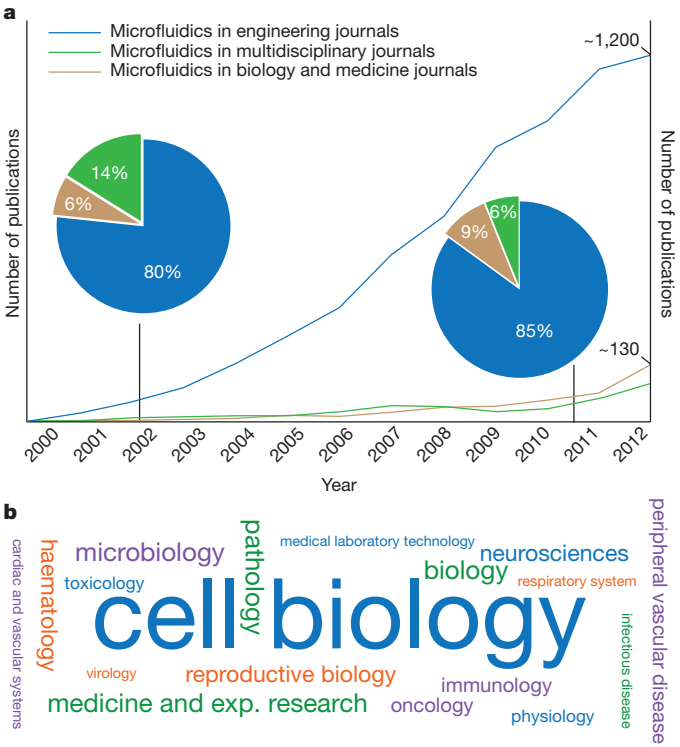


Figure 1 | Microfluidic publications in engineering, multidisciplinary, and biology and medicine journals from 2000 to 2012. **a**, In 2012, there were roughly 10 times more microfluidic publications in engineering journals compared to biology and medicine (biomedical) journals (left-hand pie chart inset). However, the share of microfluidics papers being published in multidisciplinary journals decreased as publication share in biomedical and engineering journals increased (right-hand pie chart). **b**, Word cloud illustrating what fields most frequently used microfluidics. The size of the font is proportional to the cumulative number of publications in the Web of Science (WoS) category (2000–12), with the exception of 'cell biology', which would need to be ~5 times larger. Methodology of the searches was as follows. A literature search was performed using WoS (provided by Thomson Reuters) to determine the number of microfluidics publications in various disciplines. The search was performed for the terms "microfluidic*" and "nanofluidic*". The number of publications were obtained from the WoS analytics reporting system for each search term, and then summed before being presented above. Three categories were characterized by the WoS search that capture the relevant journals for the years 2000–12. The analysis shown here as "Microfluidics in engineering journals" reports the number of microfluidic publications in the 'Nanoscience and nanotechnology' WoS category. The analysis shown here as 'Microfluidics in multidisciplinary journals' corresponds to the 'Multidisciplinary' WoS category. The analysis shown here as 'Microfluidics in biology and medicine journals' reports publications from WoS categories shown in Fig. 1b. The search explicitly excluded 'reviews', 'book chapters', 'book reviews', 'meeting abstracts', 'meeting summaries', and included 'articles'. The data shown reflects the most recent literature search, performed on 21 March 2013. The following search general string was used: Topic = (microfluidic*) AND Year Published = (2000-2012) AND Document Types = (Article) NOT Document Types = (Book OR Book Chapter OR Book Review OR Meeting Abstract OR Meeting Summary OR Proceedings Paper OR Review). This string yielded the total 'microfluidic*' publications in all WoS categories ('*' allows for permutations of the keyword). The search was then refined by the WoS categories shown above (for example, Web of Science Categories = (MULTIDISCIPLINARY). Importantly, the nominal results of this search would probably vary if other search tools such as SCOPUS, Google Scholar and PubMed were used[98,99]. For example, the gross number of publications would probably increase if SCOPUS were used for the search, as this tool indexes a higher number of journals than WoS[99].

of Sciences). The results reveal, unsurprisingly, that the overwhelming number of microfluidics papers are still being published in engineering journals (Fig. 1a). These engineering journals have facilitated the technological development and growth of microfluidics over the past decade. It is important to note that some of these 'engineering' studies may have been designed for non-biomedical purposes, but this does illustrate where the majority of microfluidic activity and exposure has occurred. Today the majority of microfluidics publications still appear in engineering journals (85%) as the microfluidics community has grown substantially, and 'biology and medicine' journals have taken some publication share from interdisciplinary journals (9% and 6%, respectively).

Last, we analysed what fields within the biomedical research community are using microfluidic technologies the most (Fig. 1b). 'Cell biology' and 'Biology' encompass most of the microfluidics publications, possibly because these categories are somewhat generic and incorporate several subcategories. Following these, the most use of microfluidics is seen in 'Haematology', 'Medicine and experimental research' and 'Immunology'. Most of these publications are for diagnostic applications (in the case of Medicine and experimental research) and the manipulation of blood samples for biology research (Haematology and Immunology)—applications where microfluidics has compelling advantages over traditional methods. However, despite these few examples, the evidence suggests that a 'killer application' that propels microfluidics into the mainstream has yet to emerge.

A case study in chemotaxis assays

The state of the art for most conventional assays used in cell biology research is evolving and improving over time. Biologists understand better than anyone the deficiencies of the techniques they use, and individual groups occasionally make modifications to traditional assays that are adopted more broadly by other biology researchers. An example of this technological evolution can be observed in visual chemotaxis assays—techniques that measure the directional migration of a cell in response to a source of chemotactic factors that change concentration in space and time.

Chemotaxis assays have improved substantially since their initial introduction in the 1960s (Fig. 2). The most widely used chemotaxis assay is known as the 'Boyden chamber' or 'Transwell' assay, developed in 1962 by Boyden[15]. The Transwell assay works by creating a concentration gradient of chemoattractant compounds between two wells that are separated by a microporous membrane. Chemotactic cells located in the upper well sense the gradient in concentration and migrate across the membrane towards the solution in the lower well where the cells are counted. Its simplicity and ease of use (no special instrumentation is required) has contributed to its widespread use over the past 50 years. Investigators have used the method to identify chemotactic factors for various cell types, despite the fact that the technique disallows observation of the cell migration path or cell morphology. This experimental limitation (along with others) led to the development of visual chemotaxis assays such as the Zigmond chamber[16]. In this system, cells can be observed as they undergo chemotaxis on a coverslip across a narrow constriction (tens of micrometres) towards a source chemoattractant. It is worth noting that the Zigmond chamber is a microfluidic device developed by biologists at least a decade before the emergence of the microfluidic/μTAS field as we know it. Importantly, this technique allows for clear imaging of cell migration and morphology. Modifications to this design, called the Dunn[17] and Insall[18] chambers, were subsequently developed, and these advances substantially improved the high-resolution, long-term imaging capabilities of visual chemotaxis assays (Fig. 2). The Insall chamber represents the most recent of a long evolution of direct-viewing chemotaxis chambers that have been developed over the course of three decades.

Microfluidics has offered many solutions for next-generation chemotaxis assays (reviewed in refs 19 and 20); however, none of these methods have seen widespread adoption at the level of the aforementioned traditional assays. Additionally, efforts to commercialize microfluidic chemotaxis assays—notable products include μ-Slide Chemotaxis (ibidi), Iuvo Chemotaxis Assay Plate (BellBrook Labs), and EZ-TAXIScan (Effector Cell Institute)—have had limited success in the marketplace. The generation of chemical gradient profiles is an area where microfluidic technologies are uniquely qualified because of the highly predictable[21], diffusion-dominant characteristics of the fluid flow at this scale (Box 1). Yet traditional assays are still predominantly used for chemotaxis studies in cell biology research. The low adoption rate of microfluidic chemotaxis assays may be due to the fluid handling expertise and infrastructure required in early designs[22,23], which may have acted as a barrier to entry for biologists[24]. Recently published microfluidic chemotaxis techniques are beginning to take usability requirements into consideration, and demonstrate simpler chemotaxis assay designs that do not require active pumping systems[25–27]. Another possibility is that biologists are more comfortable with using the existing direct-viewing chemotaxis assays that have been developed and vetted over nearly 40 years (Fig. 2). Notably, each iterative improvement on the Zigmond chamber design was published by investigators with appointments in biology (Zigmond); experimental pathology (Boyden and Dunn); and cancer research (Insall)—none of the designs were produced from 'engineering' disciplines. These technical advances were made by biologists to address unmet needs in their own research. And in the case of visual chemotaxis, the methods were, in fact, microfluidic by any reasonable definition, yet they are not typically included within the microfluidic vernacular. In the case of chemotaxis assays, engineers have sometimes erred by imposing technological complexity and functionality where it was not necessarily needed or wanted. This case study illustrates the continuing need for engineers and biologists to work closely during assay development to create usable and robust solutions that build on biologically validated approaches, while adding functionality that allows new avenues of biological inquiry.

Materials tailored for specific applications

Unlike the semiconductor industry where silicon is the backbone material on which the technology has been built[28,29], the materials used for developing microfluidic devices have undergone a large transition over the years. Early μTAS devices were fabricated from silicon[30] and glass[31] using clean-room techniques that were translated to microfluidic device fabrication. This was largely a choice of convenience (because the techniques

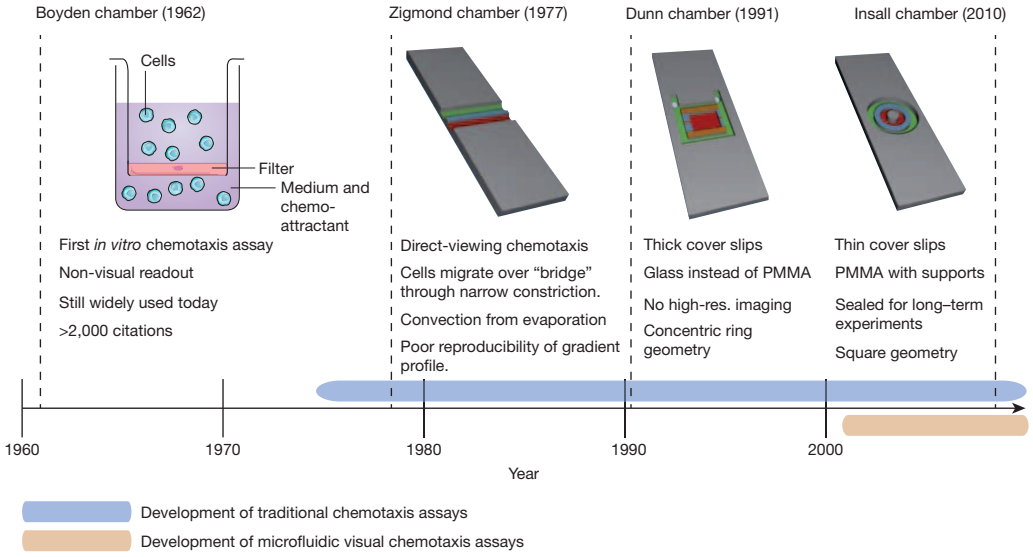


Figure 2 | The development of visual chemotaxis assays over time. The Boyden chamber assay was the first popular *in vitro* chemotaxis technique. The Zigmond chamber design has undergone several evolutionary changes (Dunn and Insall chambers) to address problems with previous versions of the assay. Note the relatively short time for which microfluidics techniques have been available in comparison to the classical visual chemotaxis assays (bars on the timeline compare the development of visual chemotaxis assays). Chamber images adapted with permission from ref. 100, Nature Publishing Group (Boyden chamber) and from ref. 18.

and facilities were already in place) and necessity (early microfluidics focused largely on electrophoretic phenomena where glass is a preferred material), but not a long-term solution for cell biology research. Silicon is opaque to visible and ultraviolet light, making this material incompatible with popular microscopy methods. Glass and silicon are both brittle materials, they have non-trivial bonding protocols for closing microchannels, and in general they require expensive, inaccessible fabrication methods. These materials were well suited for some applications (for example, electrophoresis), but were ultimately limited in their growth potential. Cheaper, more accessible materials and fabrication methods were needed to fuel the growth of microfluidic technology development and adoption.

Elastomeric micromoulding techniques were developed by Bell Labs in the 1970s[32], and first applied to microfluidics and cell biology in the 1980s[33]. In 1998, Whitesides used polydimethylsiloxane (PDMS)—an optically transparent, gas- and vapour-permeable elastomer— for the fabrication of more complex microfluidic devices[34] and helped 'soft lithography' become the most widely adopted method for fabricating microfluidic devices. It would be hard to exaggerate how important and enabling PDMS has been for microfluidics, contributing to the growth of the field in both technological development and number of publications[35]. Adoption of the material can be attributed to several key factors, including (1) the relatively cheap and easy set-up for fabricating small numbers of devices using PDMS in a university setting; (2) the ability to tune the hydrophobic surface properties to become more hydrophilic[36,37]; (3) the ability to reversibly and (in some cases) irreversibly bond PDMS to glass, plastic, PDMS itself, and other materials; and (4) the elasticity of PDMS, which allows for easy removal from delicate silicon moulds for feature replication. In addition to the practical fabrication considerations of using an elastomer, there are also useful functional advantages. Researchers have used the elasticity of PDMS to create micropillar arrays that assay the mechanobiology of various cell types[38,39]. However, perhaps most importantly, the elasticity of PMDS allows for valving and actuation[40,41], which has led to a plethora of microfluidic designs and publications. Fluidigm—the largest commercial μTAS technology company currently in the market—build their microfluidic systems using deformable elastomers (NanoFlex valves).

Despite all the beneficial properties of PDMS that enabled its rapid adoption amongst university engineers, there are several limitations to implementing the material in biomedical research. For example, PDMS has been found to leach uncrosslinked oligomers from the curing process into solution[42], requiring additional device preparation to mitigate this potentially harmful effect[43]. Additionally, PDMS has been shown to absorb small molecules[42,44], which can affect critical cell signalling dynamics. Furthermore, the vapour permeability of PDMS means that evaporation can occur in an experiment[45], which can be detrimental for cell microenvironments at micro- and nanolitre fluid volumes[46,47]. Strategies such as parylene coating the microchannel surface[48] and other techniques[49,50] have been developed to mitigate these problems, but these processes are consequences of deploying a non-ideal material for cell biology applications—the often cited 'biocompatability' of PDMS appears to be something of a misnomer. Last, the manufacture and distribution of PDMS devices to collaborators is not easily scalable, because high-throughput methods such as injection moulding, rolling and embossing cannot be used for PDMS devices. Thus, making PDMS prototypes for iterating on a new design concept is relatively easy, but making many of these devices and packaging them for collaborators or commercialization is non-trivial[51]. Given these limitations, clearly PDMS is not a one-size-fits-all material for all microfluidic applications, and particularly for cell biology research[52].

The limitations of PDMS have prompted researchers to explore alternative materials in recent years (Fig. 3). In the microfluidics community, there has been a push towards the use of thermoplastics such as polystyrene and cyclic olefin copolymer[53] for microfluidic devices (Fig. 3A), although some research laboratories have always used these materials in lieu of PDMS[54,55]. Thermoplastic materials such as polymethyl methacrylate and polycarbonate[56,57] were popular for the fabrication of μTAS

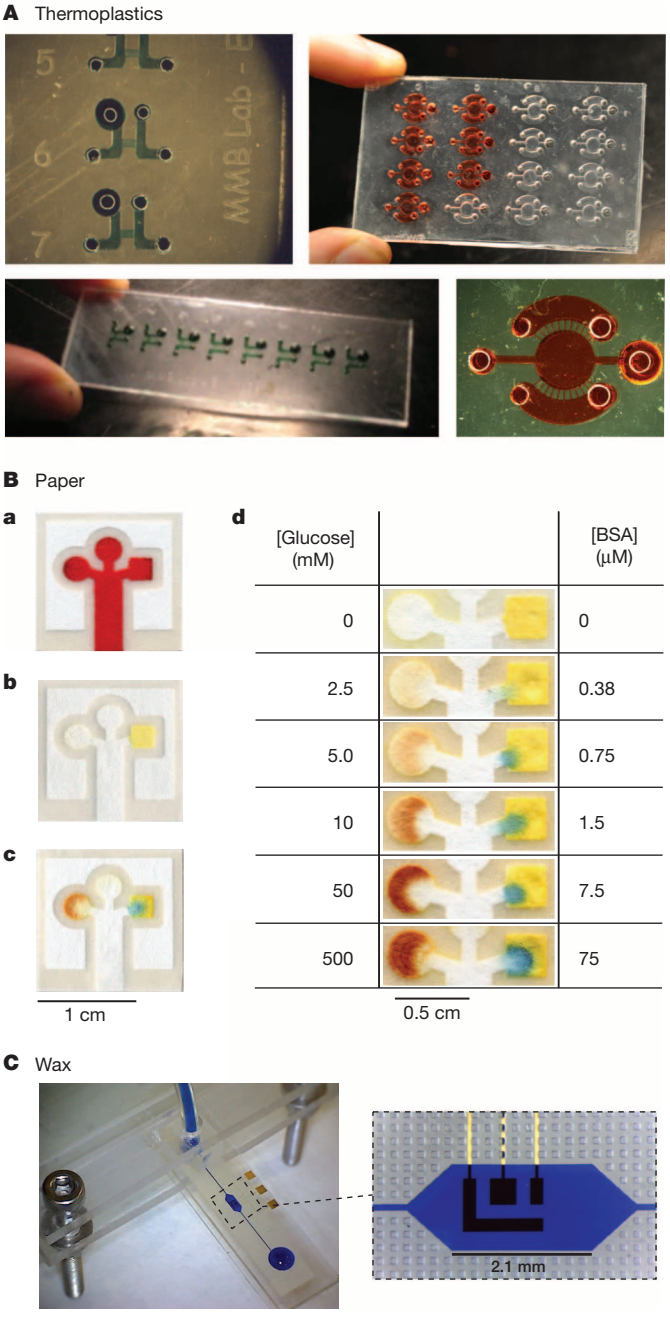


Figure 3 | Materials other than PMDS are being used for microfluidic device design. **A**, Several research groups have demonstrated accessible methods of thermoplastic microfluidic device fabrication. Examples of various microfluidic designs fabricated in polystyrene are shown. **B**, **C**, Paper (**B**), and to a lesser extent, wax (**C**) are being used in the developing world for diagnostic applications owing to benefits in device cost, operation and destructibility with limited waste infrastructure. **B**, An example of a paper-based microfluidic device for detecting glucose and protein. The integrity of the hydrophilic patterning is shown with a red dye (**a**); the detection zones for glucose (circular region on left) and protein (square region on right) are also shown (**b**); and representative tests detecting a single concentration (**c**) and multiple concentrations (**d**) of protein and glucose from an artificial urine sample are also shown. **C**, An example of a wax microfluidic device (zoomed view in inset) that can perform an enzymatic immunoassay. Figure sources, used with permission: **A**, ref. 59; **B**, ref. 64; **C**, ref. 101. **B** is adapted with permission from Martinez, A. W., Phillips, S. T., Whitesides, G. M. & Carrilho, E. Diagnostics for the developing world: microfluidic paper-based analytical devices. *Analytical Chemistry* **82,** 3–10 (2010). Copyright (2010) American Chemical Society.

devices in the 90s, but lost favour with researchers because the fabrication methods were more difficult and expensive than those of PDMS for the typical academic laboratory. However, the microfluidics community has addressed this issue by developing more accessible fabrication methods for thermoplastic μTAS devices[58–60], although these techniques are not without limitations[35,61]. We have recently argued that polystyrene should be preferred over PDMS for many cell biology applications, particularly because biologists have a long history of using polystyrene for cell culture[35]. Furthermore, the use of polystyrene mitigates or eliminates many material property issues associated with PDMS, including the bulk absorption of small molecules and evaporation through the device, and polystyrene makes handling and packaging easier for use in collaborations.

In addition to thermoplastic materials, there has been substantial progress in using destructible, cheap materials such as paper (Fig. 3B), wax (Fig. 3C) and cloth[62] for point-of-care applications in low-resource settings. These materials have the benefit of being cheap and easily incinerated[63], making them ideal choices for settings where safe disposal of biological samples is challenging[3,64]. Currently there is increasing activity in developing microfluidic paper-based analytical devices (μPADs). These μPAD devices are expansions on tried-and-tested lateral flow assays (for example, pregnancy strip test) and operate by passively wicking biological samples through patterned hydrophilic regions using capillary forces; they often use colorimetric readouts. The hydrophobic channel patterning can be accomplished using a variety of methods, such as wax printing[65], photolithographic patterning of photoresist[66], inkjet printing of PMDS[67], and flexographically printed polystyrene[68]. μPAD devices are becoming increasingly sophisticated[69,70], with a recent study demonstrating a single-step enzyme-linked immunosorbent assay (ELISA) for the detection of human chorionic gonadotropin[71].

The movement beyond PDMS with the use of thermoplastics and other materials is a positive development for the microfluidics community. Rather than solely relying on PDMS for device fabrication regardless of its limitations, researchers are beginning to consider new materials that more suitably meet the requirements of biological assays and are amendable to high-throughput manufacturing. The shift to materials beyond PDMS enables researchers to more effectively export technologies in scale, and allows for new solutions to problems in performing cell biology and diagnostic assays. However, different materials often require a re-thinking of component design. For example, it is difficult to implement the displacement valves and pumps so ubiquitous in PDMS devices in other non-elastic materials. Therefore, technological progress using alternative materials will require creative new approaches from engineers that design powerful and user-friendly μTAS devices.

When μTAS technologies are the only solution

Most of the microfluidic technologies that were developed for cell biology applications in the early 2000s sought to improve on existing macroscale assays. Many of these technologies delivered on the promised performance improvements, yet were never adopted by mainstream biology researchers. Another possible reason for this lack of adoption, beyond those we have previously discussed, is that these technologies are improvements on established techniques. Although microfluidic methods may in some cases be technologically superior, they are often only iterative improvements on methods that already exist. Someone interested in performing protein analysis might conduct a western blot or ELISA. To study cell chemotaxis, a researcher might perform a Transwell assay. To investigate tissue regeneration after a wound, an investigator might scratch some cells with a micropipette tip and see what happens. Microfluidic techniques exist that perform many of these assays with equivalent or improved performance[24], but they have not offered fundamentally new capabilities compared to the current state-of-the-art.

Within the past several years there have been a growing number of microfluidic technologies that solve problems that have not yet been addressed by macroscale approaches. Two recognizable examples that embody this distinction can be found in the glucometer and the pregnancy test (or more broadly, lateral flow assays). Each test passively wicks bodily fluids into porous materials, either blood (glucometer) or urine (pregnancy test), and performs a previously complex biochemical assay in a single step to provide an immediate measurement. Although there were benchtop assays that could perform these tasks, the portability and rapid feedback these assays provided was transformative for the end user. There are currently applications like these for which microfluidic methods have demonstrable advantages over traditional methods. These various applications share overlapping qualities that make them potentially useful techniques. However, for the purpose of this discussion, we will break them into three categories: diagnostic devices for low-resource settings; the rapid processing of biofluids for research and clinical applications; and more physiologically relevant *in vitro* models for drug discovery, diagnostics and research applications.

Diagnostics for low-resource settings

The western model of centralized laboratories processing clinical samples with expensive equipment does not translate well to the developing world. Many low-resource settings do not have the means or infrastructure to perform these tests and analyses, necessitating creative alternative solutions to meet this largely unsolved problem. Microfluidic methods are being developed to perform a variety of diagnostic tests with built-in analysis capabilities that are compatible with the infrastructure in the developing world (Fig. 4). As discussed earlier, new material systems such as paper, wax and others are being explored in this area[53,64,72]. Common themes with these devices include being ultra-simple to operate and the provision of some qualitative or quantitative output that can be measured with low-cost and ubiquitous equipment (for example, a mobile-phone camera or scanner). Also, ideally, the materials used to make the devices are easily destructible to avoid unsafe contamination, and are cheap and scalable to manufacture (preferably locally). In a recent study, Chin *et al.*[73] aimed to meet these requirements in a microfluidic chip that performs an ELISA-like assay within ~20 minutes using volumes of blood that can be obtained from a lancet puncture (Fig. 4). Importantly, the assay did not require external pumping systems; it emphasized straightforward operation; and it used cheap photodetectors for the rapid optical readout. The authors analysed more than 70 blood samples obtained from a hospital in Rwanda and successfully diagnosed human immunodeficiency virus (HIV) in all but one patient, achieving sensitivity and specificity values that rival a laboratory-based ELISA test. This study and others are promising indications that μTAS technologies could make meaningful contributions to healthcare in the developing world.

Low cost is arguably the most important feature when aiming to increase access to diagnostics in the developing world, but it is also an increasingly important factor in the developed world. If we can achieve appropriate performance/cost combinations for the developing world, many believe that these technologies will play an important role in transforming the way medicine is delivered in the developed world by enabling in-home testing and treatment. However, traditional lateral flow assays achieve a low-cost/high-performance benchmark, and thus represent a high standard against which new approaches are compared.

Rapidly assaying biofluids with microfluidics

Engineers have made use of properties unique to the microscale to enable studies that would be difficult or impossible using macroscale approaches (Fig. 5). These methods have found clinical applications, because they use ultra-low volumes of biofluids for the sample processing and can usually be accomplished rapidly and easily. To some degree these assays mimic what macroscale assays accomplish, but the methods offer new approaches that enable fundamentally new applications. For example, the rapid purification and analysis of neutrophils—the phagocytotic cells that are first responders for the innate immune system—have been demonstrated in several studies in recent years for clinical and research applications[26,74,75]. Importantly, these techniques reduce blood processing times from roughly an hour (using millilitres of blood from a venipuncture[76]) to a few minutes (using only microlitres of blood from a finger prick). Thus, the methods can be applied to measure neutrophil

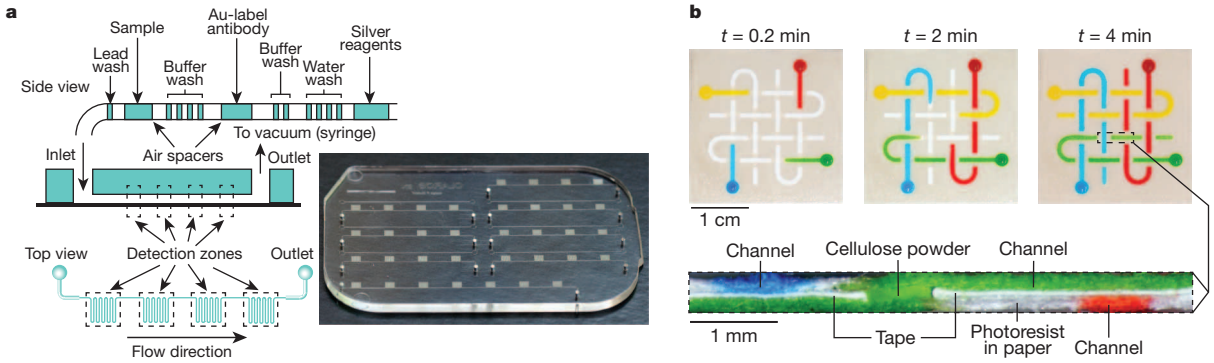


Figure 4 | Diagnostics in the developing world. These are excellent examples of exploiting the benefits of μTAS technologies where classical (Western) diagnostic paradigms fail. **a**, A user-friendly cartridge to perform enzyme-linked immunosorbent assays (ELISAs) for the diagnosis of HIV and other diseases. A schematic showing the functional steps of the assay is shown on the left and the microfluidic device is shown on the right. **b**, 3D μPAD showing complex fluid handling operations that occur passively in a paper device for diagnostics in resource-limited settings. The example shows the flow of several coloured dyes in a patterned μPAD device, with a cross-section of the 3D structure also shown. Figure sources, used with permission: **a**, ref. 73; **b**, ref. 64. **b** is reprinted with permission from Martinez, A. W., Phillips, S. T., Whitesides, G. M. & Carrilho, E. Diagnostics for the developing world: microfluidic paper-based analytical devices. *Analytical Chemistry* **82,** 3–10 (2010). Copyright (2010) American Chemical Society.

function for diagnostic and research purposes, enabling a new class of studies that have previously been beyond the capabilities of macroscale methods[26]. Other purification schemes have been developed that take advantage of the increased dominance of surface tension at the microscale to sort target analytes in biofluids across multiphase barriers (for example, oil and water; see Box 1) using fast and simple procedures[77,78]. Not only is this purification scheme simpler and faster than most macroscale methods, but improved sensitivities for protein and genetic purifications may be achievable owing to a reduction in the number of wash cycles required to carry out an experiment. These applications are only some of the examples where microscale benefits are being used to perform experiments that are not reasonably achievable using macroscale techniques.

More physiologically relevant *in vitro* models

The pharmaceutical industry is currently faced with unsustainable research and development (R&D) costs[79,80] that require it to change how the development and approval of new drugs are pursued[81–83]. The industry faces multiple headwinds, such as the exclusivity on blockbuster drugs soon expiring for several companies, and dramatically fewer new drugs being approved by the Food and Drug Administration (FDA) in recent years. These circumstances necessitate new strategies for drug development that increase R&D productivity in order to avoid a potential drought in effective new drugs coming to market.

Microfluidics researchers are taking aim at this problem by developing potentially transformative technologies to mitigate the cost of new drug development. A new class of microfluidic devices seeks to replicate *in vivo* organ function on a microchip (Fig. 6). This new class of so called 'organ-on-a-chip' technologies integrates several well-understood microfluidic components into a single *in vitro* device, allowing researchers to more closely recapitulate *in vivo* function (both normal and disease states). This ambitious effort is still in its infancy, though several promising studies have developed examples of these biomimetic systems. Examples of organ (or disease)-on-a-chip technologies include gut-on-a-chip[84], lung-on-a-chip[85], blood vessel-on-a-chip[86–88], cancer-on-a-chip[89–91] and kidney-on-a-chip[92]. Furthermore, these modular systems could theoretically be combined into a complete 'human-on-a-chip' model that mimics *in vivo* function of these organs working in concert[93]. The result would be a class

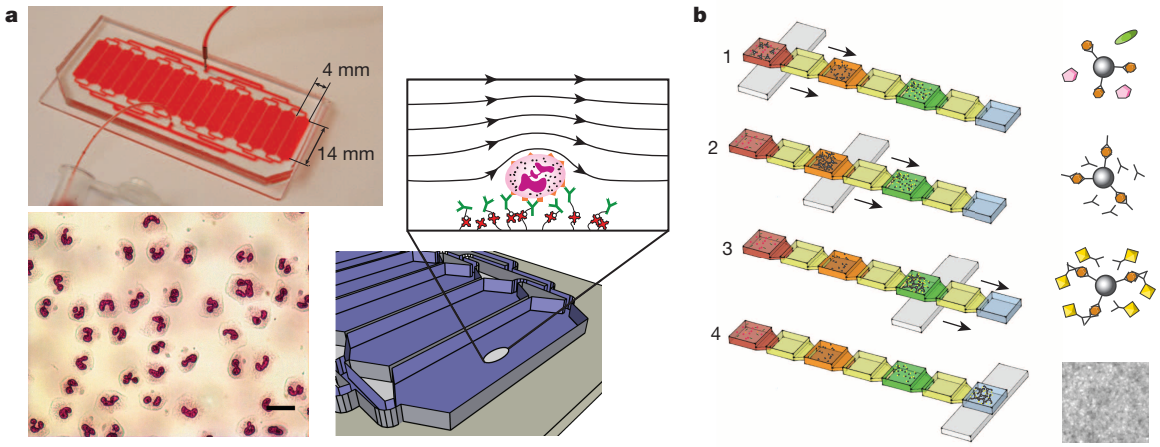


Figure 5 | Rapid purification microfluidic systems. **a**, A microfluidic device to purify neutrophils within minutes using antibody-based capture for subsequent diagnostic or research analysis. The microfluidic device is shown at the upper left with stained neutrophils that have been sorted from whole blood below (scale bar = 20 μm); an illustration of the neutrophils captured within the microchannels by antibodies (zoomed view in inset) is also shown. **b**, A technique to purify target analytes such as RNA, cells and proteins by simply sliding a magnet across an immiscible aqueous–oil interface. An example shown here illustrates four steps to purify protein from a sample (zoomed view to the right shows detail) by (1) removing the analyte bound to paramagnetic particles across the first aqueous-oil barrier, (2) binding a primary antibody to the analyte and dragging it across another aqueous-oil barrier, (3) binding a fluorescently labelled secondary antibody to the complex and bringing it across another aqueous-oil barrier into the imaging well (4), where the fluorescence is measured to detect the amount of analyte (white and grey fluorescent image). Figure sources, used with permission: **a**, ref. 74; **b**, ref. 10. **b** is adapted with permission from Berry, S. M., Maccoux, L. J. & Beebe, D. J. Streamlining immunoassays with immiscible filtrations assisted by surface tension. *Analytical Chemistry* **84,** 5518–5523 (2012). Copyright 2012 American Chemical Society.

of sophisticated *in vitro* assays with which drugs could be tested, in the hope of increasing the predictability of a new drug (that is, hit rate) before animal testing (possibly even replacing animal trials) and human clinical trials. In a tangential application, blood vessel-on-a-chip devices have already been used for the diagnosis of sickle cell disease in the clinic[86,94]. For example, Tsai *et al.*[86] described a microfluidic chip that recapitulated *in vivo* conditions of a blood vessel—such as blood flow rate, endothelial cell shear stress and biochemical activation states—in order to reliably detect vascular occlusions due to sickle-cell disease. This system highlights how certain properties of microfluidic systems, such as high-resolution micropatterning and precise control of the haemodynamic and shear profiles in the microchip, enabled the measurement of biophysical abnormalities in a clinical setting. Much more work is still required before organ-on-a-chip methods can be adopted in mainstream drug R&D, although early developments in this area are promising. Indeed, AstraZeneca—a multinational pharmaceutical company—has recently announced a collaboration with Harvard's Wyss Institute to research the integration of organ-on-a-chip technologies into their drug development.

Where we go from here

The question of how to increase the adoption of microfluidic technologies in mainstream biomedical research remains largely unanswered, and we argue there are no guaranteed routes to achieve adoption. We have shown that microfluidic technologies are being used for some studies in biology research and diagnostic applications; however, the large majority of microfluidics publications are still in technical journals specific to the field (Fig. 1). Adoption of new technologies that supplant or even complement existing methods is often a slow process. For evidence of this, we consider the computer mouse, which took 20 years to appear in the Macintosh computer after its invention by Engelbart in the 1960s. But this does not mean microfluidics engineers should become disillusioned or discouraged. Researchers in the field must develop deliberate and thoughtful strategies that will best push the technology forward. We now have several decades of experience to draw on, and there are some useful lessons we can apply.

Fostering mutually beneficial collaborations

During the early years of microfluidics, the field did not have a successful strategy for transferring technological developments to non-engineering users. Perhaps the idea was that researchers from the biology community would rush to work out how to make use of these new technologies. Clearly this formula of engineers and biologists leading separate academic lives does not benefit either community. Fortunately researchers have acknowledged that a divide between the developers of the technology and the end-users is counterproductive. Most of the recent microfluidics papers published in 'Biology and medicine' journals are co-authored by engineers, biologists and clinicians. This evidence of increasing collaboration is a promising development for everyone involved. In order to sustain this trend, microfluidic researchers should court collaborators from biology and clinical laboratories (and vice versa). Direct interaction and feedback from the end-user is tremendously beneficial during technology development. Furthermore, new applications and ideas can be generated from biology collaborators that engineers—being non-experts in cell biology or clinical research—would never have considered.

The simplest solution is almost always best

All the signs indicate that there is no simple solution for accelerating the adoption process; however, there are design choices engineers can make in order to lower the barrier to entry for biologists. How the end-user interacts with a new technology is a critical aspect of whether the method is adopted. Microfluidics engineers have been attempting to simplify fluid handling challenges in their designs with passive pumping approaches that only require a micropipette to operate[8,25–27,77,78,95]. Additionally, some have explored the use of centrifugal forces to perform complex assays using a 'lab-on-a-CD' design[96]. Many microfluidic applications require the use of external pumps and pneumatic fluid handling systems; examples include most organ-on-a-chip devices and techniques that require continuous flow to generate specific shear profiles (for example, biomimetic blood vessel models). However, engineers should limit the use of these external systems whenever possible. Creating a simpler approach often requires more creative solutions, but this can greatly improve the

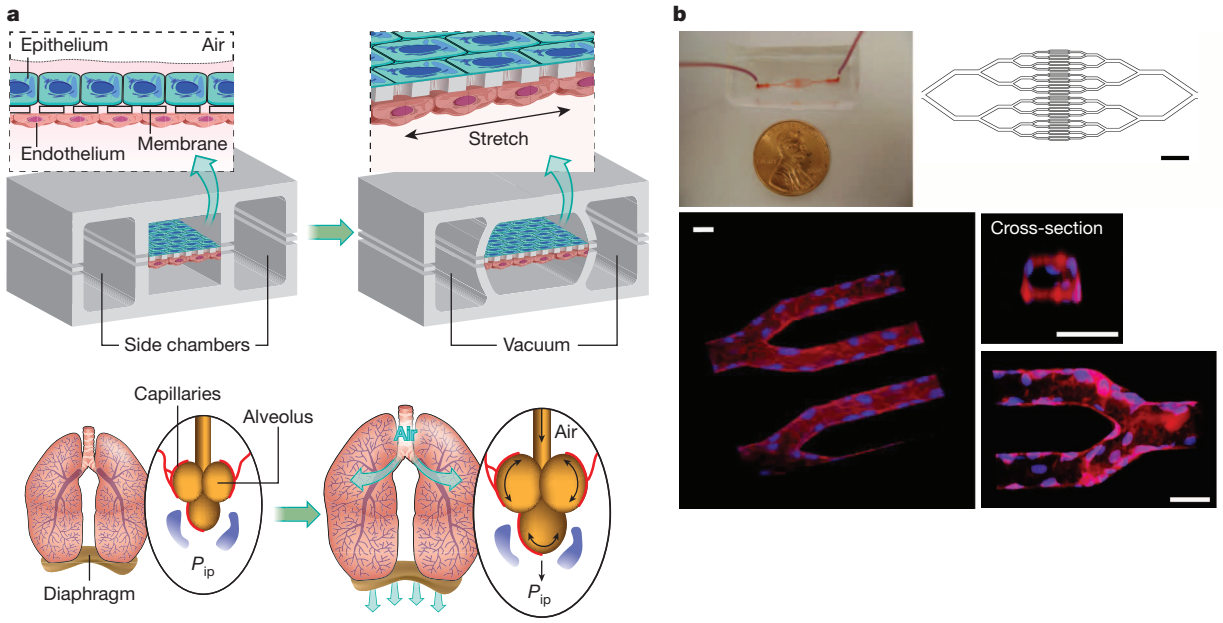


Figure 6 | Organ-on-a-chip assays for drug development and specialized diagnostic applications. **a**, Complex microsystems can be developed to recreate an organ's physiology, such as the physiology of the lung, directly on a microfluidic device. The diagram illustrates a biomimetic microfluidic design that actuates stretching of tissue in a breathing-like manner by using vacuum in side chambers to strain the cell-coated PDMS membrane. This process mimics the reduction in intrapleural pressure (P_{ip}) in the lungs during breathing. **b**, Biomimetic blood vessel and capillary networks can also be recreated *in vitro* to diagnosis SCD and other diseases involving blood vessel–whole blood interactions. An image of the microfluidic device is shown (top) next to a penny for scale, with a diagram at right showing the increasingly narrow capillary network; confocal microscopy images of the endothelial cell-lined lumens within the device are also shown (bottom) with the cell nucleus (blue) and cell membrane (red) visible. Scale bars: 600 μm (black), 30 μm (white). Figure sources, reprinted with permission: **a**, ref. 85; **b**, ref. 86.

experience for the end-user. Paper diagnostic assays are an excellent example of single-step, automated and user-friendly μTAS solutions where the technology is not visible and the user can focus on interpreting the results[64]. We have recently developed a similarly straightforward, automated approach for general cell biology applications that does not require external pumping equipment or even a micropipette to perform complex assay protocols[97]. General problems of packaging and distributing microfluidic technologies to collaborators will also need to be addressed until microfluidic assays become more commercially viable in the academic research market. These problems should be viewed through the lens of user-friendly assay design.

Finding the right problems to solve

The case study we have used (chemotaxis assays) helps to illustrate how competing technology platforms continue to improve over time as microfluidic technologies develop. Some of the touted advantages of microfluidic systems that existed 20 years ago are not as stark today because technological improvements have been made to more traditional and widely accepted assays, often narrowing the initially perceived performance advantage of microfluidic solutions. This evolution in the technology landscape highlights the need for finding the right problems in biology and medicine to solve with microfluidic approaches. For example, microfluidic solutions have advantages over many technologies for diagnostics in the developing world. However commercializing these technologies is challenging because, by definition, the desired diagnostic devices will not generate much revenue or profit. So the breadth and depth of impact may be great for this particular application, but a disconnect exists between development and commercialization. Likewise, there may be niche biological questions that can be addressed using microfluidic methods, but for which broad commercial markets do not exist. A key consideration in the development of new microfluidic methods in academic research should be whether the use of microfluidics introduces truly enabling functionality compared to current methods. When a potential application passes this test, the chances of contributing useful technology to the field are substantially higher.

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