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Review

Small but Perfectly Formed? Successes, Challenges, and Opportunities for Microfluidics in the Chemical and Biological Sciences

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Microfluidic systems are pervasive in many areas of experimental science, but what are the real advantages of this technology? We describe some of the features and properties that make microfluidic devices unique experimental tools. In addition to pointing out some of the less effective uses of this technology, we assess the most successful applications of microfluidics over the last two decades and highlight the areas where they had the greatest impact. We also propose applications where microfluidic systems could be applied to the greatest effect in the future.

INTRODUCTION

A microfluidic system is an engineered fluidic device where flow is non-turbulent and thus highly ordered. Normally, this coincides with critical dimensions of the device in the order of tens to hundreds of micrometers. The term microfluidics, however, describes a set of techniques that has expanded its influence from early application in the analytical sciences and ink-jet printing[1] to problems in the complex syntheses of novel materials,[2] ultra-high-throughput biological assays,[3] and the simulation of entire organ systems.[4]

Early predictions regarding the potential of new technologies are notoriously unreliable and often turn out to be incorrect. Indeed, if a technology is an enabling set of techniques rather than a stand-alone product, such predictions are almost impossible to make. A cursory survey of the literature validates the impression that microfluidic technologies are increasingly being used as component tools in basic science. Although many dedicated journals exist to disseminate core microfluidic research exploring the physical basis of fluid behavior at the microscale and in circuit-level designs, a rapidly growing number of studies that use microfluidic tools appear in journals covering natural or medical sciences. Accordingly, it is timely to assess what microfluidic technology is currently used for, when it is used to its best effect, and where, in the future, it stands to do the most good.

THE MICROFLUIDIC TOOLSET

Contemporary assessments of microfluidics often propose fields that are best set to benefit from the technique. These include genetic analysis,[5] capillary electrophoresis,[6] DNA amplification,[6] clinical chemistry, cell-based assays and cellular analysis,[5,7] single-cell analysis,[7] proteomics,[5,6] point-of-care (PoC) diagnostics,[5] drug discovery,[5] and small-molecule and nanomaterial synthesis.[5,8,9] However, comparison of the early and current scope of core microfluidic journals illustrates how

The Bigger Picture

Interest in microfluidic technologies has been driven by concomitant developments in fields such as genomics, proteomics, drug discovery, high-throughput screening, and diagnostics, as well as a recognized need to perform rapid and efficient experiments on small-sample volumes. Today, the relevance of microfluidics in these fields is significant and characterized by a range of features that accompany system miniaturization. Put simply, microfluidic activities have been stimulated by the fact that physical processes can be more easily controlled when instrumental dimensions are reduced to the micrometer scale. Although the microfluidics toolset has influenced many areas of science, where has its impact been greatest, and where has it now faded? What scientific disciplines will be transformed by microfluidics in the future, and what challenges remain? In this review, we aim to answer these questions.

significantly the areas of interest and impact have changed. Although in the early 2000s, the focus lay on "synthetic chemistry," it later shifted toward cell-based systems and more generally "biochemical experimentation". This transition came naturally considering that many biochemical and physiologic systems operate on the microscale and are thus well suited to microfluidic interfacing or simulation. Similarly, microfluidic technologies enable modern day biologists and chemists to work efficiently at single-cell and single-molecule levels, which was not possible before.

Defining devices as microfluidic solely through their scale is as simple as it is unsatisfactory. It is more appropriate to describe microfluidic systems in terms of their performance and behavior. Indeed, although numerous disparate effects reveal themselves when moving from macroscale to microscale dimensions, some critical features driven by the scale dependency of heat and mass transfer are particularly important. First, the small instantaneous volumes associated with microfluidic environments dictate that mass transport is almost always dominated by diffusion. This means that fluidic mixing occurs in a controllable fashion and laminar flow regimes are fully developed. In addition, the large surface-area-to-volume ratios typical of microscale environments ensure thermal homogeneity across the system and rapid heat transfer between the device and the contained fluid. A device operating within these parameters can be considered to be microfluidic. It is important to highlight the practical consequences of this: (1) laminar, predictable fluid flow with high-velocity gradients and dominant interfacial effects (allowing for the controlled manipulation of fluid streams, inertial effects on particles without fluid turbulence, and the generation of monodisperse emulsions); (2) compartmentalized picoliter fluid volumes that can contain single entities and concentrate reaction products; and (3) uniform reaction conditions to control reactions in bulk or on surfaces. Within this broad definition, microfluidic systems split into two subclasses, which operate in different ways and with varying characteristics and advantages. In our discussion, we define microfluidic systems as single-phase (operating with solely an aqueous or non-aqueous fluid) or multi-phase (operating with two or more fluidic phases in contact, for example, aqueous-fluorous or liquid-gas phases).

Most analytical systems have a temporal component built into the process, and thus any ambiguity in defining a starting point in time will generate uncertainty in the result. The rapidity and uniformity of mixing events within a range of microfluidic formats means that such temporal variability can often be kept to a minimum, which manifests itself as increased precision in analytical measurements[10] or a decreased dispersity in product characteristics.[11,12] Similarly, many chemical and biological systems are temperature dependent and variations in local temperatures lead to disparate outcomes. Within planar-chip microfluidic formats, localized hot (or cold) spots are extremely uncommon.

On the basis of such advantageous features, microfluidics appears almost too good to be true. So why is the contemporary laboratory still dominated by bench-top analytical instrumentation? And why, after almost three decades of intensive research into microfluidics, do we find few if any standardized microfluidic tools within the experimentalist's toolkit? Several fields of research, which at one time were deemed important, have led to outcomes with limited real-world impact. For example, in the early years, fluids within microfluidic formats were commonly motivated via electroosmotic forces.[13] The reasons for this were partly historical, in that much early microfluidic research focused (to excellent effect) on transferring

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http://dx.doi.org/10.1016/j.chempr.2017.01.009

electrokinetic separation techniques from macroscale to chip-based formats. (We note that a number of systems and instruments incorporating electrokinetic microfluidic components have been successfully commercialized and adopted by bioanalytical end users over the past decade. These include products marketed by Agilent, Caliper, GE Healthcare, Shimadzu, PerkinElmer, and Illumina.) In such systems, electroosmosis (the bulk movement of a conductive solution past a stationary surface as a result of an externally applied electric field) is of undoubted utility because fluid velocity does not vary with radial position across the flow path, moving parts are unnecessary, and flow is insensitive to back pressure. However, when assessed as a pumping modality alone, its limitations are apparent; electroosmosis can pump only polar liquids, can attain maximum volumetric flow rates of a few microliters per minute, and is easily disrupted by temperature variations and gas evolution. Accordingly, from a contemporary perspective, electroosmotic motivation is most suited to specific applications such as analytical separations and sample pre-treatment, rather than as a general fluid manipulation tool.

Nevertheless, many microfluidic tools are of undoubted utility, and we first highlight key areas where microfluidic tools had significant effect and then propose applications where microfluidic technologies might have the most dramatic impact in the short to medium term. Naturally, this analysis cannot be exhaustive, but we hope it will be informative. To do this, we subdivide the field into topics that share key techniques or key limiting phenomena.

GENOMICS

A noteworthy application area for microfluidics is genomics, and this case is instructive in understanding the drivers of success. Economics of scale and throughput are important factors in genomics, where there is an insatiable appetite for measurement, and the scale of experimental design is limited only by reagent cost and time. Microfluidics provides a natural solution to this problem through the miniaturization and automation of nucleic acid biochemistry in high-throughput formats. Small-volume analysis can also fundamentally improve the sensitivity of genomic analysis, enabling experiments with very limited templates (see below). Accordingly, by scaling reactions down to nanoliter or picoliter volumes, microfluidics simultaneously provides throughput and economy while also enhancing performance (Figure 1).[14]

Although the potential technical advantages of microfluidics in genomics were recognized almost two decades ago, success is equally a product of outside factors. Most notably, next-generation sequencing has increased the raw throughput of reading DNA sequences by over six orders of magnitude,[15] providing a strong stimulus for faster, cheaper, and more sensitive sample preparation. Sequencing has also brought genomics to an increasingly dominant position in medical research and diagnostics, creating a need for high-performance and/or PoC analytical technologies with ever-improved sensitivity and precision. Finally, genomics has been a major driver for the commercialization of microfluidics, greatly amplifying the impact of these approaches.

COMPARTMENTALIZATION FOR SINGLE-MOLECULE AND SINGLE-CELL ANALYSIS

In microfluidic systems, the ability to compartmentalize molecules or cells within small and isolated compartments serves as a key driver enabling both single-cell and single-molecule measurements. The simplest articulation is a robustly physical

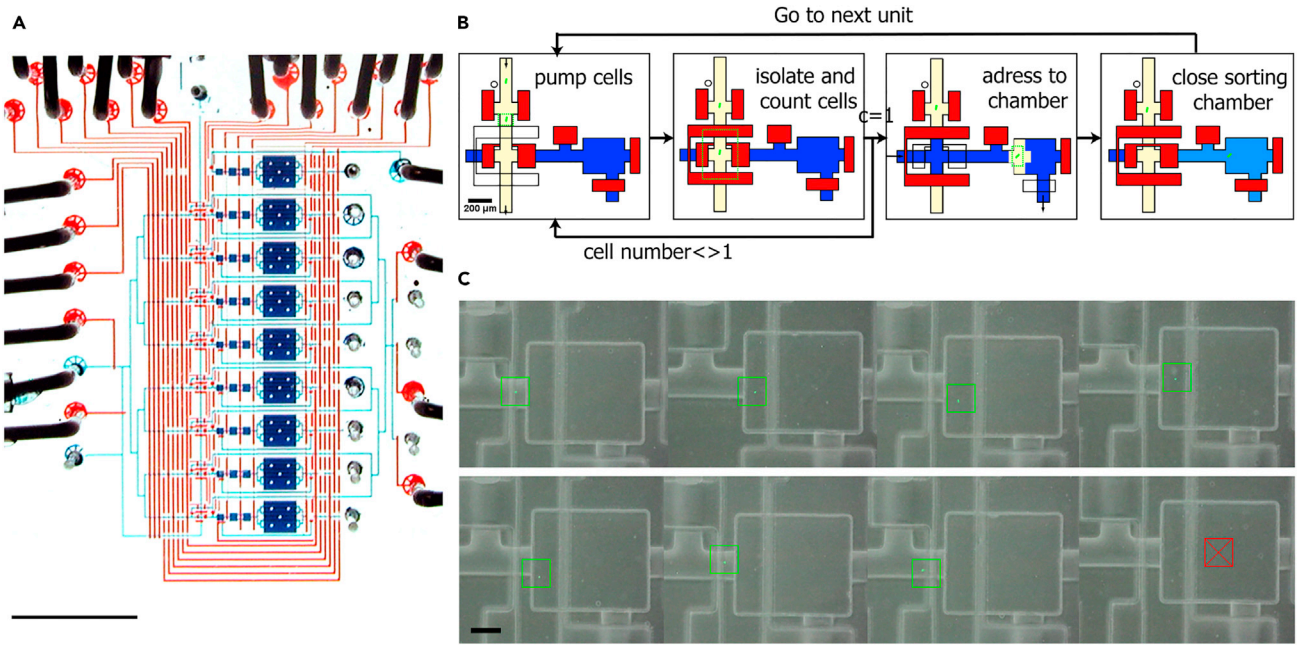

Figure 1. Single-Cell Isolation and Genome Amplification Chip

(A) Photograph of the chip. The channels and chambers are colored blue, and the control lines (to actuate valves) are colored red. Scale bar, 5 mm.

(B) Schematic drawing of the cell-sorting procedure. Closed valves are red, open valves are transparent, and cells are visualized in green.

(C) Exemplary results of cell sorting. The cells are marked with green squares. Seven chambers contain cells, and the chamber with a red crossed square is an empty control chamber. Scale bar, 100 µm.

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approach; trapping small volumes of fluids within physically segregated containers controlled by structures or valves. Conversely, fluids can be compartmentalized and processed as droplets within an immiscible carrier fluid to form segmented flows. Significantly, once formed, these droplets can either be maintained in flow for serial processing or retained in structures to enable parallel readout.[16] These approaches allow an extraordinarily large number (>10^6) of experiments to be performed simultaneously in an addressable array-based format. Importantly, by subdividing a sample with an appropriate concentration of cells or molecules into droplets, one can encapsulate a single entity within each isolated volume with droplet occupations that obey Poisson statistics.[17] Following the results of single-cell studies, it is now widely appreciated that cell-to-cell variability is an important feature of many biological systems. This variability arises from multiple sources: limitations in using cell-surface markers to purify, or even to define, different cell types; asynchrony in cellular responses; differences in chromatic state; and stochastic variations that affect gene expression or cellular response. The ability to reach a "single-entity" limit for large sample sizes is critical in biological investigations to prevent rare species from "getting lost" during the ensemble averaging that occurs in assays such as PCR, western blots, or ELISA.[17] Such effects are not normally a result of strict enhancement of a given reaction but occur rather through the suppression of noise. For example, detection of a single molecule in a conventional (e.g., microwell plate) 10-µL volume is exceedingly difficult because of unwanted reaction side products or contamination. When performed in a 10-pL, volume these effects are reduced by six orders of magnitude, making single-molecule detection extremely reliable. Indeed, compartmentalization is now the foundation of many emerging technologies that operate on single molecules or cells in parallel: digital PCR, digital ELISA, and a

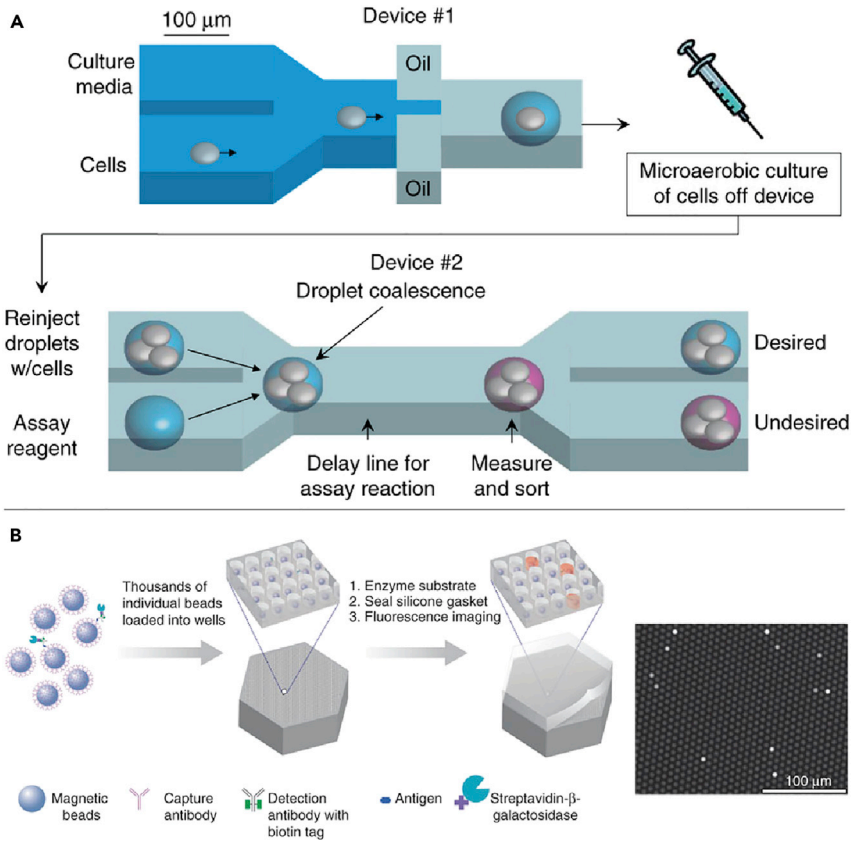

Figure 2. Two Examples of Single-Cell or Single-Molecule Analysis Platforms

(A) Mixing of cells with culture media to form droplets, which are subsequently collected in a syringe and cultured in an incubator. After culturing, single-cell-containing droplets are united with droplets containing fluorescent enzymatic assay reagents for quantification of the extracellular concentration of the metabolite of interest. Depending on the fluorescent signal, cells are sorted into desired or undesired populations. Reprinted by permission from Macmillan Publishers Ltd: Nature Biotechnology (Wang et al.[103]), copyright 2014.

(B) Digital ELISA platform for single-protein detection. Microscopic beads capture single proteins that are subsequently labeled. Beads with or without protein are loaded into femtoliter-volume wells together with enzyme substrate. The relative number of wells exhibiting fluorescence corresponds to the concentration of protein in bulk solution. Reprinted by permission from Macmillan Publishers Ltd: Nature Biotechnology (Rissin et al.[104]), copyright 2010.

variety of single-cell analysis platforms (Figure 2).[18] In fact, commercial microfluidic instruments based on these concepts now provide widely available solutions for biological research and potentially diagnostics.[18] Single-cell analysis provides a direct route to dissecting heterogeneity and opens a new frontier for biological study. As a result, digital PCR has now become the gold standard for precision nucleic acid quantification in high-value applications such as diagnostics and seems poised to replace qPCR as a routine laboratory tool.

Recently, molecular biology methods, including multiplexed PCR, whole-genome amplification, and whole-transcriptome amplification, have advanced to the point of making large-scale single-cell genomics a practical reality. A common feature of these methods is that reactions are assembled by consecutive addition of reagents without the need for purification steps. This is readily implemented in valve-based microfluidic formats, allowing for integration of cell capture, processing, and nucleic acid amplification. Indeed, valve-based microfluidics have

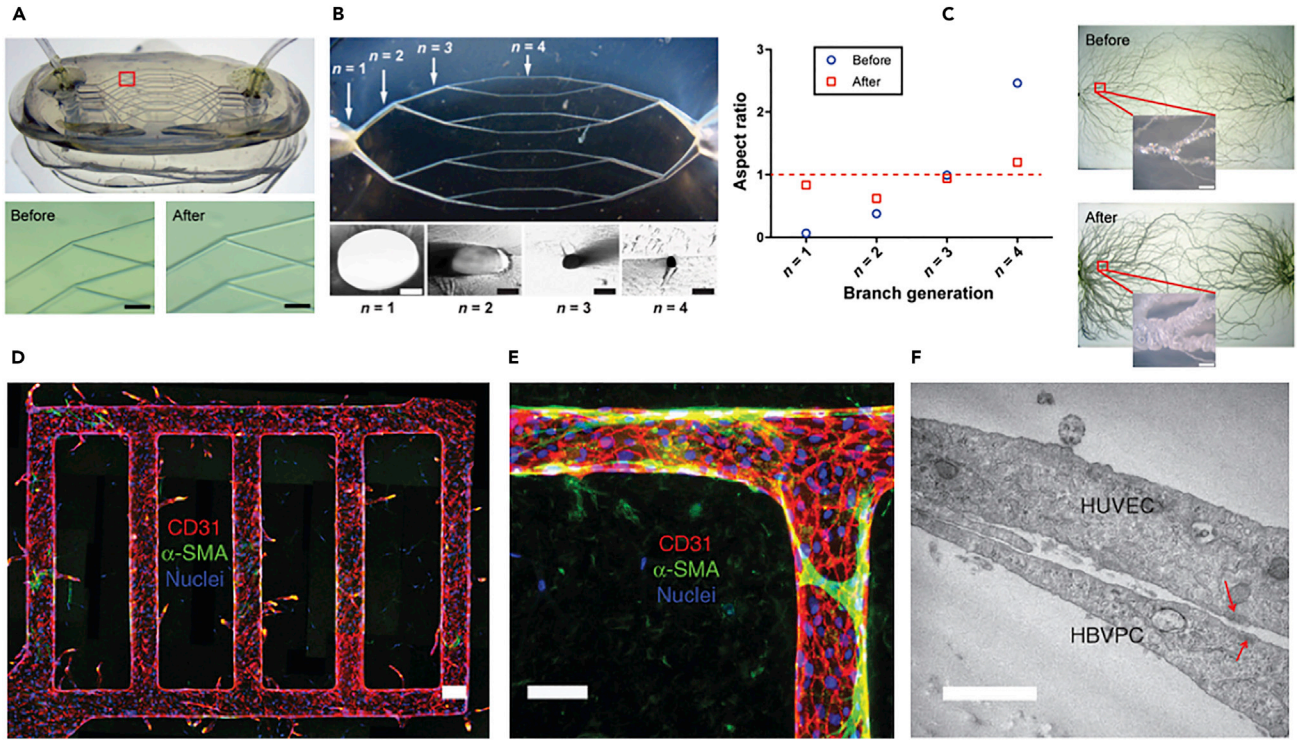

Figure 3. Microfluidic Networks for Vascular Modeling

Two approaches to addressing problems of vascularization within microfluidic networks.

(A–C) The generation of circular branched channel networks via photolithography or electrostatic discharge followed by inflation to achieve vascular-like structures. The modification by inflation allows for a circular venous-type profile and a more realistic aspect ratio, avoiding sharp corners, which invoke abnormal cell differentiation. (A) A pseudo-3D network constructed from a seven-layer stack of planar PLA branched networks before and after expansion (scale bars, 300 μm). (B) Because of the limitations of lithographic microfabrication, the initially rectangular large channels in early branch generations have smaller aspect ratios than those in later generations. The degree of circularity (i.e., when the aspect ratio approaches unity) is simultaneously improved across all branch generations after a single expansion step (15 psi of pressurized air for 20 min at 80°C; white scale bar, 500 μm; black scale bars, 100 μm). (C) A 3D branched microchannel network embedded in a 1.5 × 5 × 8 cm molded PLA block by electrostatic discharge contains a distribution of microchannel diameters that are not optimal for cell seeding (upper image). After air expansion (lower image), average diameters are significantly increased throughout, and the sidewall topology becomes smoother (scale bars, 500 μm). Reprinted from Huang et al.[22]

(D–F) Formation of microvascular networks in vitro. The preformed channel network is populated with heterogeneous cell types, which organize and grow in a manner analogous to neoangiogenesis. (D) Endothelial cells (HUVECs) respond to stimulation in the presence of cells (human brain vascular pericytes [HBVPCs]) seeded in the matrix by sprouting new branches, as visualized by confocal microscopy. (E) Smooth muscle cells seeded in the matrix associate with the endothelium, as visualized by confocal microscopy. (F) Ultrastructure of the cellular interfaces formed between HUVECs and HBVPCs, including a deposited layer of basal lamina (red arrows), can be visualized by transmission electron microscopy. Staining: CD31, red; DAPI, blue; α-SMA, green (D and E). Scale bars, 100 μm (D and E) and 1 μm (F). Reprinted by permission from Macmillan Publishers Ltd: Nature Protocols (Morgan et al.[105]), copyright 2013.

been applied to a variety of single-cell analyses including qPCR, whole-transcriptome amplification,[19] whole-genome amplification of mammalian and bacterial genomes,[20] long-range haplotyping,[21] and digital PCR.[17] Generally, nanoliter volume analysis has improved assay performance and also offers key advantages of improved cell handling and the direct visualization of cells before processing.

MIMICKING PHYSIOLOGIC SYSTEMS

The body in basic terms incorporates a diverse array of microfluidic components, because many essential processes, such as oxygen and nutrient transport, threat detection, and homeostasis rely on blood capillaries with diameters between 5 and 10 μm. An obvious area where this idea applies is in the vascular system. Unsurprisingly, a range of venous-inspired structures have been formed with the use of microfluidic architectures (Figure 3).[22] These studies have primarily looked at the

problems of network design and how this affects and potentiates tissue growth, but also at the assembly of biomimetic cell layers to line these networks, which gives insight into embolization[23] and the venous formation process.[24] One of the key roles of arterial and venous architecture is the maintenance of oxygenation, and this has formed the basis of microfluidic investigations of vascular architectures for artificial tissues. More recently, platforms have also been developed to model hypoxic damage in cardiac tissue during heart attacks.[25]

Cells grow differently in biological settings. Key differences between standard in vitro culture on plastic surfaces and in vivo conditions include (1) the spatial arrangement of cells in defined architectures, (2) the presence of multiple cell types that communicate through contact and soluble factors, and (3) spatial and temporal variations in physical conditions such as shear stress, strain, and pH. Microfluidic architectures are ideal for patterning and growing artificial tissues and organs and also for monitoring responses of individual cells to various stimuli.[26] This arises because microfluidic systems can be patterned at a variety of scales and flow regimes, just like living tissue, but generally confined to two dimensions, allowing for easier interrogation and study. Currently, several microfluidic-enabled culture systems that partially mimic physiology (the liver in particular) are commercially available and significantly are being evaluated for improved prediction of pharmaceutical response in human cells before expensive clinical trials.[7] Use of these systems is likely to radically reduce the need for animal models in future drug development, as immortalized cell lines in an on-chip format will be easier to use and have far fewer ethical issues.

Biomimetic systems can also help us understand how tissues grow and develop. This process is often complex, relying on multiple inputs such as strain and chemical microenvironments. Microfluidic systems have been developed to look at problems as diverse as how neutrophils navigate through tissues,[27] how confinement affects neurite development,[28] and how the local hydrodynamic environment affects tumor growth.[29] There are many aspects of cell behavior that are poorly understood, not least of which relates to fluid flow inside cells. Work on giant cells suggests that microfluidics is the ideal way to study this.[30]

EXPLOITING FLUID PHYSICS TO FIND NEEDLES IN HAYSTACKS

The increased throughput and experimental confidence afforded by microfluidic tools is well illustrated through the analysis of rare events: finding a needle in the proverbial haystack. Via conventional experimental techniques, rare events must generate signals of sufficient intensity (or contrast) to be detectable above a background. As already noted, one approach to elucidate rare events is to controllably break the sample up into smaller and smaller pieces and interrogate each one separately. Alternatively, microfluidic tools can be used to concentrate rare species above the common background. This is achievable because the laminar nature of flow (at the low Reynolds numbers typical in microfluidic systems) ensures that particle and fluid motion is well defined and can thus be engineered for purpose. For example, the ability to direct streams of cells to surfaces allows trapping and concentration based on surface-specific antigens, whereas the ability to direct cells to specific streamlines on the basis of size, electrical properties, or deformability allows selective concentration based on physical properties.[31] This behavior contrasts with macroscale environments, where the ability to control fluid and (cell-sized) particle motion is almost always hindered by the chaotic motion of the flow. On the microscale, however, this is not the case, and we now highlight two direct beneficiaries of this.

Circulating Tumor Cells

The most comprehensive models of cancer metastasis incorporate a significant focus on circulating tumor cells (CTCs) as a key factor in the spread of the disease to multiple tissues and organs. However, interest in such cells goes much further because they provide an alternative to repeated biopsy, which can be of paramount importance not only in gathering data for population-scale therapies but also in tailoring specific treatments to the individual.[32]

Typically, as few as 1–5 CTCs are present in a 1-mL blood sample containing 10^9 erythrocytes and 10^6 leukocytes. Accordingly, CTC analysis is a tremendous challenge in terms of separation, concentration, and detection. Moreover, to reliably harvest CTCs, a significant volume of blood must normally be processed (in a high-throughput manner) with extreme selectivity (i.e., high purity). The problem is, therefore, one of discrimination, i.e., how does one selectively capture CTCs over other cells. Methodologies for this are discussed elsewhere,[33] but two main categories emerge: those using affinity-based systems and those using physical parameters to affect separation. In the case of affinity capture systems, several microfluidic strategies have isolated CTCs with success based on selective cell affinity chromatography with controlled shear-stress-dependent adhesion between capture surfaces and cell surface markers.[34] Although these approaches reduce processing rates (10–50 mL of whole blood per minute) to avoid detaching captured cells from surfaces with overly intense shear stresses, they lead to capture based on unique molecular markers, which can yield higher efficiencies for some cancer types.[35] Indeed, the very best of these systems utilize both methodologies to increase purity and throughput.[36]

So why are microfluidic tools for CTC analysis potentially so important? First, the separation and purification efficiency attainable by a controlled microfluidic approach is decidedly better than for conventional (gold standard) systems that mix cells randomly with magnetic beads.[36] This results in the isolation of a much larger number of cancer cells (1–100 per mL) by microfluidic approaches than by the gold standard CellSearch clinical test (0–50 per 7.5 mL).[37] Second, size- or inertial-based separation systems have a key advantage; they do not rely on affinity. In size-based systems, the CTCs are filtered from other blood contents by their relatively larger size. Inertial-based systems rely on the migration of the CTCs across streamlines in a channel induced through inertial forces. Affinity systems by their nature require the development of specific antibodies or capture agents for a particular cell population. In other words, they catch only what you are expecting or trying to find. Size-based systems not only capture larger CTCs regardless of surface chemistry but also do not modify the captured cells, which remain untagged or unbound to surfaces. This is of key importance when considering the need for downstream cell culture and analysis. Of course, size-based approaches are unable to separate (smaller) CTCs, which are of a similar size to leukocytes (<12 μm in diameter) and could represent a varying fraction of the entire CTC population depending on cancer type.[38] Regardless, CTC capture and analysis has been a growth area in microfluidics and is one where the relationship with cancer researchers truly enables new working modalities.

Blood Microbe Culture

In sepsis, the analysis of the microbes circulating in blood is a key step to successful treatment. It is, however, surprisingly difficult to find these microbes. Typically, one can expect a false-positive rate between 5% and 50% and a true-positive rate between 15% and 30%.[39] The typical microbial burden in blood is similar to or lower

than that of CTCs, even in advanced sepsis, which dictates that current best practice is to culture from blood (effectively amplifying the signal) and then test for antibiotic susceptibility of the culture-forming organisms. This approach has two key drawbacks. First, the culture time is typically between 12 and 36 hr, which is often untenable because sepsis is a medical emergency and can rapidly lead to death. Second, the culture step is particularly susceptible to adventitious organism contamination.[40] What is needed, therefore, is a method by which rare pathogenic microbial cells can be separated from the overwhelming burden of other cells present in blood. As described previously, microfluidic systems are uniquely suited to screening for rare cells. This suggests application of the same basic methods to the separation and identification of microbes or activated immune cells and screening bacteria for antibiotic susceptibility without the need for extensive culturing.[41] Indeed, a combination of these steps would allow for a rapid clinical decision with the potential to completely change clinical practice in sepsis. We also note that the high efficiency with which microfluidic systems can isolate bacteria or activated immune cells from blood raises an intriguing possibility. It has been proposed that improvements in survival rates of patients with severe sepsis can be achieved by reducing the bacteria or abnormally activated immune cell load in blood.[41] Microfluidic blood processing has the potential to do this, i.e., capture pathogenic cells selectively and return undamaged blood cells to the patient.[41] Such a dialysis-like therapeutic would reduce systemic immune activation, preventing further organ damage if initiated early enough in the disease process. In this respect, it should be noted that commencement of such a therapy would depend on an effective diagnostic test that is predictive of patients progressing toward severe sepsis. Microfluidic approaches have been successful in addressing similar diagnostic challenges as we now discuss.

RAPID AND COST-EFFECTIVE DIAGNOSTICS

The potential of microfluidics to create an integrated miniaturized and stand-alone laboratory has long been a dream of the community. This capability is particularly pertinent in PoC or low-resource settings where functional laboratories are simply not available. Despite efforts over two decades, this goal is largely unfulfilled. Indeed, rather than a "lab on a chip," most experiments still involve putting a small microfluidic device on a sizable instrument, such as a fluorescence microscope, or interfacing it with macroscale control architecture. This situation is changing, however, and some truly portable systems are beginning to be realized and, more importantly, commercialized.

Unsurprisingly, the first systems to emerge employed microfluidic cartridges that were inserted into small transportable instruments suitable for field use.[42] To achieve true portability, however, all functional components and detection systems should be miniaturized. For example, much effort has been dedicated to shrinking optical components such that they go from occupying a lab bench to a form factor that matches the fluidic system by taking advantage of rapid advances in optoelectronics,[43] as exemplified by sophisticated but robust and cost-effective smartphone cameras.[44] In contrast, electrochemical sensing provides for a detection method that is inherently miniaturized and low cost, although somewhat more limited in terms of sensitivity, limits of detection, and applicability.[45]

A good illustration of the state-of-the-art in commercial PoC instrumentation is in HIV diagnostics for use in developing countries.[46] These instruments usually utilize some type of microfluidic cartridge for sample preparation and manipulation but still rely on external electronics and instrumentation for measurement and control. For

example, some microfluidic cartridges use antibody-coated channel systems for capturing CD4-positive cells to facilitate downstream imaging[47]; others take advantage of microfabrication to create channel systems able to align cells for detection without the need for sheath flows.[48] These portable instruments are now being translated and tested in low-resource settings to quantify CD4 lymphocytes for HIV diagnostics,[46] although in most cases, such instruments still require significant upfront investment.

Genetic analysis procedures (which we discuss elsewhere) such as PCR and immunoassays are also routinely translated into microfluidic cartridges that fit into portable instruments for PoC diagnostics. Although the goal of having affordable diagnostics to address pressing healthcare problems in developing countries is a key driving force in this area, some microfluidic diagnostic or prognostic tests are also being developed as lab scale instruments for the developed world. These instruments typically take advantage of the fluid-handling and shaping capabilities offered by microfluidics and the isolation and enumeration of individual cell types as previously described.

Finally, a discussion of microfluidic-based diagnostics, especially in the context of low-resource settings, is not complete without mention of paper-based devices,[49–53] the most notable of which are dipstick and lateral-flow formats. Paper-based diagnostics often utilize colorimetric assays so readout can be done visually; with the disadvantage that visual readout cannot be quantitative. As a result, significant efforts are also being spent to incorporate low-cost, quantitative detection schemes into paper-based formats. These include the assimilation of electrochemical detectors[54] and integration of smartphone cameras for readout given their ubiquitous nature even in developing countries.[55] Paper-based microfluidic devices uniquely offer the potential for truly disposable, self-contained, and low-cost diagnostic devices (Figure 4).[56] Yet, without the aid of external instrumentation, minimalistic paper-based diagnostics are yet to fully overcome technical challenges, such as adequate sensitivity and accurate quantitation.[57] Additionally, certain assays, such as PCR, cannot be translated into a fully self-contained paper-based format. Nevertheless, many smart assay designs have been implemented into the paper microfluidic format, and several tests, such as those for HIV and influenza, are already commercially available, with more sure to come.[49]

MAKING THE MOLECULE YOU WANT

Microfluidic reaction systems have a long history, which has been documented in detail elsewhere.[58] Briefly, the benefits associated with performing chemistry in microfluidic reactors are significant and a result of the scale-dependent processes of heat and mass transfer. This gives rise to a number of advantages, including the ability to process reduced reagent volumes, improved selectivity, the acceleration of mass-transfer limited reactions, small reactor footprints, enhanced safety, and facile routes to scale-out that have made the technology attractive as a synthetic tool. Unsurprisingly, microfluidic reactors have found significant academic and industrial application in the synthesis of nanomaterials,[59] natural products,[60] and a range of small molecule drugs and pharmaceuticals,[61] where their ability to generate products of exceptional quality in a direct and robust fashion is well recognized. However, a key lesson learned from over two decades of research in this area is knowing when to apply a microfluidic solution to a given problem. An instructive example of this idea is in the synthesis of radiochemicals. Radiotracers for positron emission tomography (PET) represent a challenge of particular timeliness to the synthetic chemist.

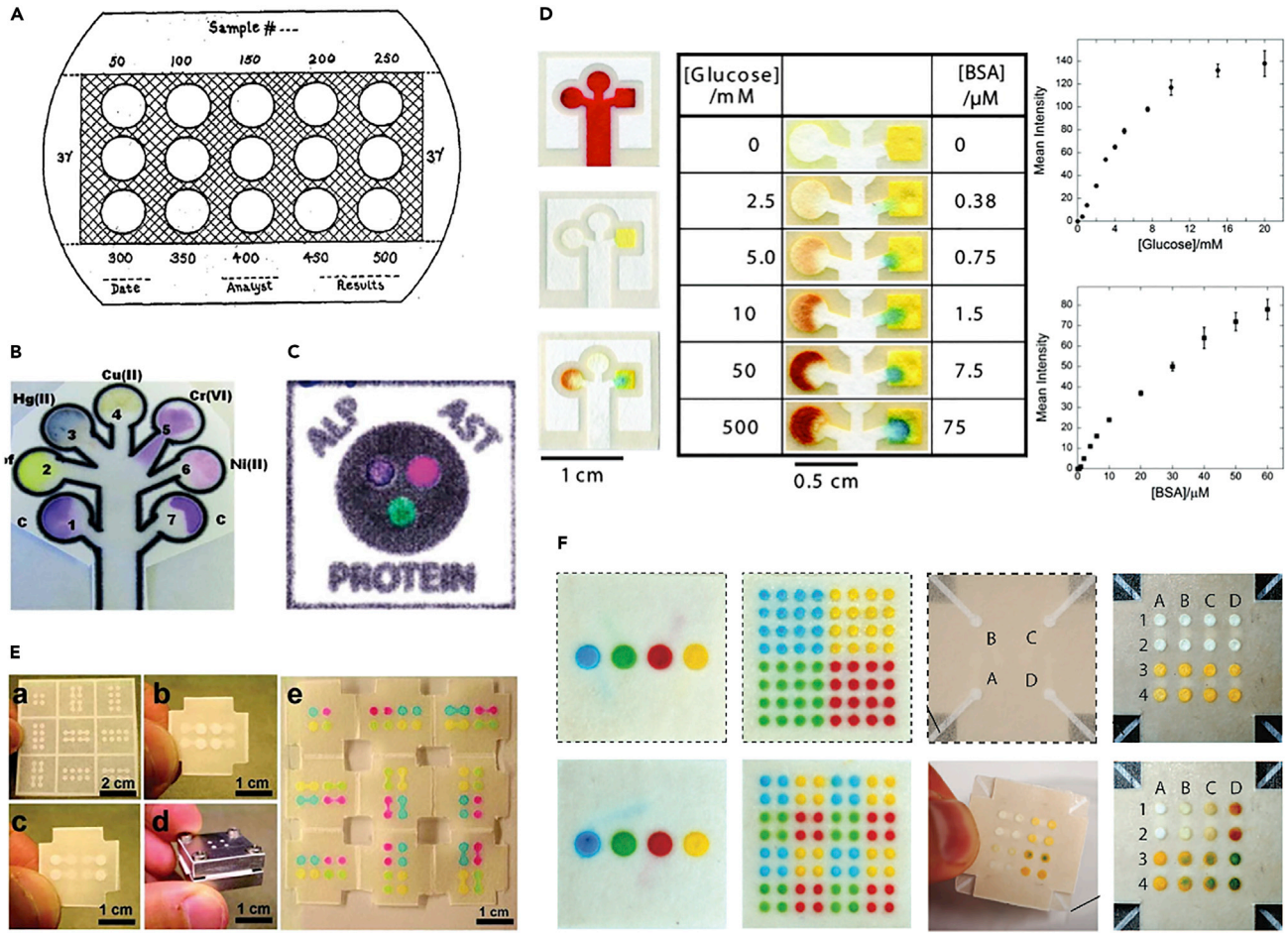

Figure 4. Paper-Based Microfluidics

(A) Schematic of an early paper-based diagnostic device where water-repellent zones are formed in filter paper by paraffin printing. Reproduced from Yagoda.[51]

(B) Paper-based microfluidic device for the analysis of heavy metals incorporating colorimetric detection. Sample is added to the circular regions of assay zones 3–6, after which lateral flow is performed with water. Reprinted with permission from Hossain and Brennan.[57] Copyright 2011 American Chemical Society.

(C) Paper-based microfluidic device that measures two enzymatic markers of liver function (alkaline phosphatase [ALP] and aspartate aminotransferase [AST]) and total serum protein. Reprinted with permission from Vella et al.[56] Copyright 2012 American Chemical Society.

(D) Microfluidic paper-based analytical device for the analysis of glucose and protein in urine. Calibration plots for the concentration of glucose and BSA in artificial urine are generated by a desktop scanner. Reprinted with permission from Martinez et al.[50] Copyright 2009 American Chemical Society.

(E) A 3D paper-based microfluidic device assembled according to the principles of origami. Chromatography paper contains photolithographically patterned channels, reservoirs, and a folding frame. Reprinted with permission from Liu and Crooks.[52] Copyright 2011 American Chemical Society.

(F) 3D paper-based microfluidic devices that distribute four samples added to the fluid inlets on one layer into arrays of 64 test zones on another. Such devices are used for the detection of glucose and BSA. Reprinted from Martinez et al.[53] Copyright 2008 National Academy of Sciences.

Their intrinsically short half-lives mean that they are fiercely radioactive and decay quickly. Consequently, their synthesis must be performed within a shielded environment and in a rapid fashion to retain as much activity as possible for diagnostic use. Because PET tracers are produced in small amounts (typically on a per-patient basis), the microfluidic approach becomes extremely attractive. Starting with ^{18}F targets such as fluorodeoxyglucose (^{18}F-FDG)[62] and ^{18}F-fallypride[63] and moving toward the more challenging arena of ^{11}C targets,[64,65] microfluidic systems have had an increasingly successful impact on the synthesis and diagnostic use of PET tracers (Figure 5).

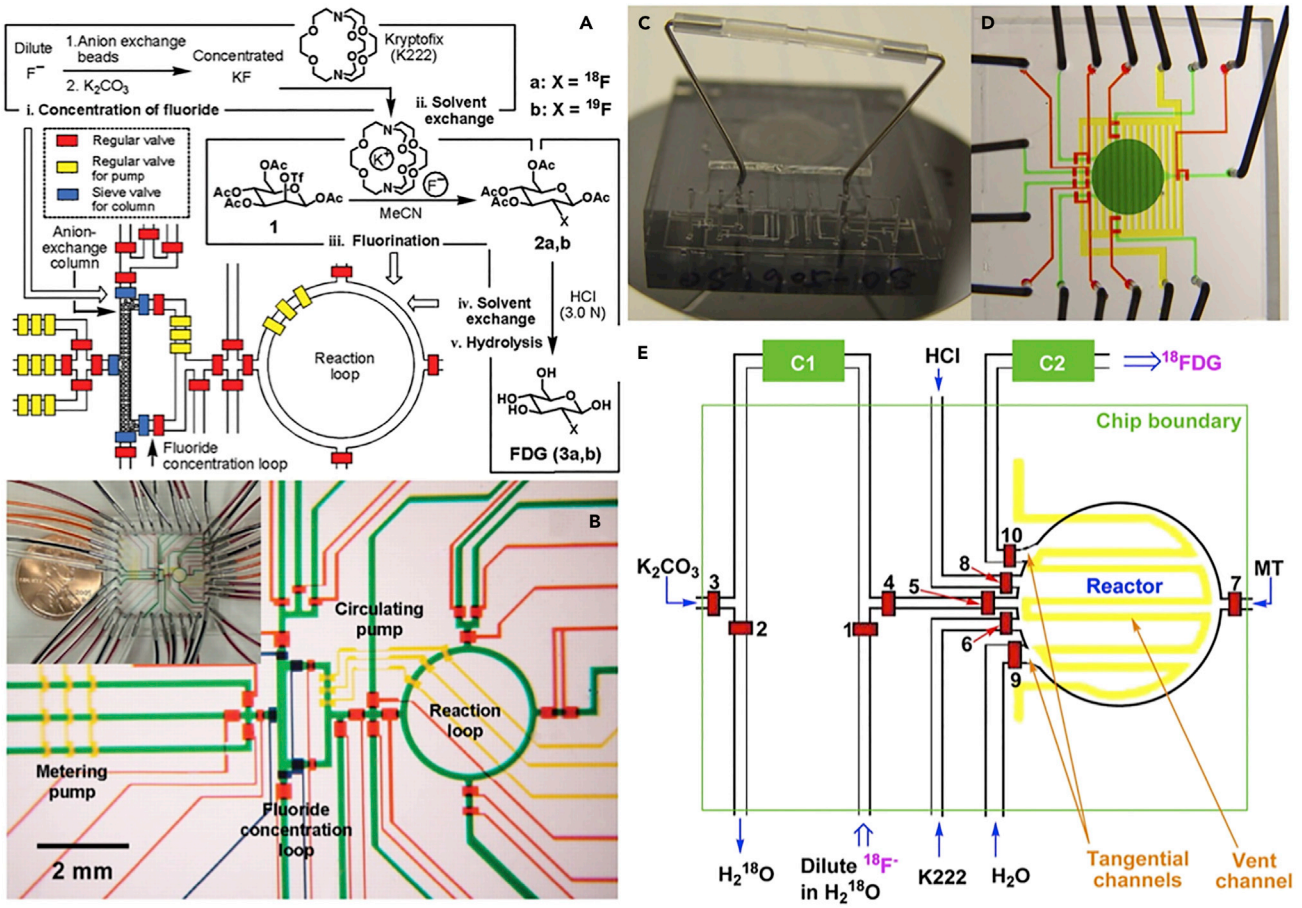

Figure 5. Microreactors for Radiotracer Synthesis

(A) A schematic representation of a chemical reaction circuit used in the production of 2-deoxy-2-fluoro-D-glucose (FDG). Five sequential processes are indicated: (i) fluoride ion concentration using a miniaturized anion exchange column, (ii) solvent exchange from water to dry MeCN, (iii) fluorination of the D-mannose triflate precursor **1**, (iv) solvent exchange back to water, and (v) acidic hydrolysis of fluorinated intermediates (**2a** or **2b**) in a ring-shaped reaction loop. Nanogram amounts of FDG (**3a** and **3b**) are produced. Circuit operation is controlled by pressure-driven valves, whose delegated responsibilities are illustrated by color: red for regular valves (isolation), yellow for pump valves (fluidic metering circulation), and blue for sieve valves (trapping anion exchange beads in the column module).

(B) Optical micrograph of the central area of the circuit. Microfluidic channels are loaded with food dye to assist visualization; colors are as in (A), plus green for fluidic channels. Inset: actual view of the device; a penny (diameter, 18.9 mm) is shown for comparison.

(C) Image of a microfluidic device with an off-chip ion exchange column controlled by on-chip valves.

(D) Image of a chip designed for coupling with an off-chip ion exchange column operated by off-chip loop valve. Microfluidic channels are filled with dyes to aid visualization. The fluid network is shown in green, valves in red, and the vent in yellow.

(E) Complete design of a coin-shaped reactor chip. C1 is an ion-exchange column, and C2 is a product purification column, both located off-chip. Reagents are introduced or removed through indicated channels, via open valves 1–10. A radiator vent is shown in yellow. The light green square outlines the physical boundaries of the chip. K222, Kryptofix2.2.2.

Panels (A) and (B) are from Lee et al.[62] Reprinted with permission from AAAS. Panels (C)–(E) are reprinted from Elizarov et al.[65]

CONTINUING CHALLENGES

Materials

A detailed assessment of material limitations on microfluidic technology is well beyond the scope of the current discussion. Suffice it to say that no one material is suitable for every application, and accordingly each microfluidic system must be made with an end use in mind. The advent of soft lithography to fabricate devices in the elastomer polydimethylsiloxane (PDMS) catalyzed microfluidic research growth in the early late 1990s. Indeed, many contemporary laboratories use nothing

else because of the low technology and investment threshold required to structure with micrometer-scale resolution. Despite its widespread use, PDMS has many limitations and is ill suited to mass production. Conversely, glass or silicon substrates are robust but require sophisticated fabrication processes whose cost or access is prohibitive to many.[58] Interestingly, both silicon and glass are emerging as important materials for nanofluidic devices comprising high aspect ratio channels, integrated electrodes, and e-beam-patterned nanoscale features.[66] That said, currently available fabrication methods have largely limited microfluidic devices to quasi-2D planar formats. Several groups have used 3D printing, for example, to print 3D microfluidic reactors and reagents in situ, but these remain beyond the grasp of most. Both the resolution and price of consumer-grade 3D printers have been plummeting over the past 5 years and are now at a point where they are beginning to be applied to microfluidic device fabrication. Direct printing will not only greatly expedite device prototyping but also give scientists an easily accessible and empowering tool to express their creativity—much in the way that the introduction of PDMS soft lithography did 15 years ago.

Modular Components

During the industrial revolution (and even earlier with the printing press), the idea of mass manufacturing of modular parts that could be assembled into more complex systems and interchanged at will became widespread. The logic was that parts could be exchanged between systems and that updates to the larger system could be achieved without re-engineering unchanged components. Interchangeable parts of course require standardized interconnections (e.g., screws and bolts with defined geometries and threading). This paradigm reached a completely new level during the microelectronics revolution, in which modularity was introduced at several levels. At the level of circuit boards capacitors, resistors, transistors, sensors, microprocessors, and other circuit elements are abstracted to "black boxes" with defined input and output responses and seamlessly connected and interchanged to perform logical operations, with standardized wiring on the breadboard connecting components. This same paradigm has also been applied to microfluidic systems with mixed success.

Analogous microfluidic "breadboards" with embedded fluidic "wiring" and fluidic circuit elements have been developed for continuous flow microfluidics[67] and are commercially available,[68] however differences between how electrons and fluids containing reagents behave has limited the widespread use of such systems. First, although metal wiring has negligible resistance and capacitance, either high fluidic resistances (small channels) or large dead volumes (large channels) become issues within the breadboard itself, leading to loss of reagents, particles, or cells between components and/or the accumulation of large pressure drops. Second, although the arrangement of flowing electrons in a wire does not affect the function of logical operations, the arrangement of fluid parcels in a flow that is deformed or disturbed upon passing through larger (i.e., higher Reynolds number) interconnects will severely affect downstream function. Nevertheless, both single-phase and multiphase microfluidic platforms have benefitted from modularity of design, but currently these approaches require device-specific modifications for assembly into a functional system. Many fundamental components such as droplet generators, splitters, and mergers have been developed for droplet platforms,[16] whereas other components such as gradient generators, flow focusers, particle separators, and valves have been demonstrated for continuous-flow systems. Unlike traditional electronic circuits, however, these components are not assembled on a breadboard, and the design for each component and connection requires the engineer to make

custom calculations for the entire system (e.g., droplet splitting to equally sized smaller droplets requires balanced downstream resistances).

The level of modularity and abstraction that has been applied to the design of monolithic integrated circuits, in which custom software allows the assembly of components and quick prediction of the outputs of the entire system, could benefit the design of monolithic microfluidic devices in the future. Software such as *Spice* or *Modern Electronic Design Automation* enables circuit designers to easily iterate and predict the behavior of an integrated circuit before costly fabrication, without knowledge of detailed semiconductor physics for each transistor.[69] The trade-off here is that the designer is then limited to a subset of well-understood transistor geometries and design rules. Software packages that abstract difficult-to-simulate fluid physics to obtain the input-output response for a subset of fluid channel geometries are now in their infancy but are moving toward similar functionality for arrangements of pre-computed fluidic circuit elements and could benefit the future microfluidic designer.

Miniaturization: Is the Advantage Transferable?

Across all fields of technology, miniaturization has long-term cost advantages as a result of lower material and energy consumption; however, critical new capabilities also arise when scaling down. The reduction of operating scale allows exploitation of unique micro- or nanoscale physics, which can enable the researcher to tackle problems previously deemed insurmountable. In nanotechnology, this idea is nicely illustrated when downscaling bulk gold or silver to nanoscale particles. This transition causes optical absorption and emission properties to change dramatically because surface electron density dominates behavior.[70] Similarly, in microfluidics, exploitation of unique scale-dependent effects is often critical for eventual impact and use. Low Reynolds number operation while maintaining relatively high velocities and Péclet numbers (that assess the ratio of advective to diffusive transport rates) allows the shaping of reagent flow streams and the manipulation of particle, droplet, and cell motion without turbulence or diffusive blurring, something almost impossible to achieve in larger channels with aqueous solutions. In addition, relatively small viscous (capillary number, *Ca*) or inertial (Weber number, *We*) forces in comparison with interfacial forces allow for the precise "dripping" of drops with uniform size.[71] Small volumes of these "micro-droplets" or valved chambers that contain single cells or molecules allow discretized readouts as previously discussed. In contrast, just using microfluidic plumbing to valve and route flows from one place to another—directly scaling down larger-scale fluid handling—has had value but limited utility.

Although flows with low Reynolds number are achievable without scaling down, uniquely in microfluidic systems, low Reynolds number conditions are readily obtained with aqueous solutions and with appreciable downstream velocities that enhance convective transport and velocity gradients. This regime of laminar flow, besides avoiding turbulence (one source of blurring of co-flowing streams), also reduces diffusive blurring because of the higher Péclet numbers than those achievable in larger channels operating at the same *Re*. Avoiding both blurring effects allows for stable co-flowing streams of different reagents without walls, yielding for example precise spatial stimulation at the cellular scale.[72] Further, such well-behaved flows are advantageous when combined with other unique physical affects that arise in this regime of finite *Re* and larger velocity gradients, such as inertial flow deformation[73] and inertial lift forces on particles and cells.[74] The velocity difference across a structure in the flow deforms the flow substantially, enabling control of fluid

cross-sectional shape. Moreover, velocity differences across a particle lead to lateral migration, precise positioning, and separation of the particle based on size, shape, or deformability.[75]

When scaling down (cross-sectional) system dimensions to tens or hundreds of micrometers, interfacial tension and surface forces between immiscible fluids dominate gravitational forces and normally viscous or inertial forces from the flow. This leads to several unique capabilities, including controlled breakup of fluid jets to droplets with diameters comparable with the underlying channel as a result of an interfacial (Rayleigh-Plateau) instability, as well as the ability to guide droplets along surfaces by modifying surface tension locally with electric fields (electrowetting) or merge droplets controllably with electric fields.[76] Solid-liquid surface tension dominating over gravity or viscous effects also leads to appreciable capillary flow in porous structures such as paper. The dominance of surface tension is also connected to the larger surface area to volume ratios associated with microscale dimensions. An example of when a larger relative surface area plays a unique role is in the mediation of an oxygen layer that quenches polymerization around microchannel surfaces and enables particle fabrication without adhesion to the channel surface.[77] Furthermore, large surface areas are ideal for capturing cells[78] or molecules[79] with high efficiency. Besides facile mass transport, heat transfer from or to surfaces is facilitated and enabling for chemical reaction control. In addition to fluid-originating forces, other force fields scale favorably in microfluidic systems. Electrodes patterned in close proximity lead to higher E fields with lower operating voltages, useful for on-chip dielectrophoresis, electroporation, electrophoresis, and electroosmosis.[80] Similarly for magnetic fields, local microscale magnetic materials of microscale dimensions lead to stronger magnetic field gradients and enhanced forces.[81]

EMERGING APPLICATIONS

Processing Living Organisms and Tissues

Microfluidic structures are characterized by dimensions that match well with the size of single cells, and for some cell types with long cellular projections, such as neurons, different portions of the same cell can be independently addressed by a suitably designed microfluidic system. For example, in neuronal cell cultures where it is desirable to individually access the cell body and axonal processes, a simple fluidic design connecting a large channel (hundreds of micrometers wide) to a series of small channels (with widths of a few micrometers) oriented orthogonal to the large channel will allow the cell body of the neuron to be placed in the large channel while preventing it from physically from entering the small channels into which the cellular processes grow.[82] Accordingly, such a device enables differential fluidic access to the neuronal cell body and axon. Moreover, refinement of such designs has been used to isolate organelles localized to the axons from those in the dendrites and cell bodies.[83] In addition to physical barriers, microfluidic networks have also been developed to create molecular gradients that guide cell migration, growth, and connectivity.[84] Cells develop and self-assemble into functioning networks as a result of the chemical and biological cues they sense, and microfluidics, with its exquisite ability to shape solution environments both locally and over distance, is proving to be an excellent platform for creating tailored microenvironments for cellular systems.

Placing neurons in microfluidic devices often means that they must be dissociated from tissue and then cultured within a fluidic device. But in many situations, it is necessary to study neural communication and function in the context of an intact

tissue. Here, microfluidic designs have also emerged as powerful tools to create localized microenvironments for dosing tissues with cellular and even subcellular resolution.[72] In one design, called a microfluidic probe,[85] a circulating flow is generated between adjacent outlet and inlet channels that form the "tip" of a pipette, thereby creating a small self-confining volume as a result of the laminar nature of fluid flow and ineffective diffusive transport. Such devices are rapidly making inroads in shaping and controlling microenvironments of cellular dimensions to facilitate the study of network function over entire tissues and activities in this area can be expected to grow rapidly in the short term.

Another emerging application of microfluidic systems is the processing and analysis of living organisms. Model organisms such as *Drosophila melanogaster* and *Caenorhabditis elegans* have played a significant role in understanding a range of biological phenomena such as neuronal development, cell differentiation, gene regulation, and apoptosis. Their importance as basic tools in molecular biology lies in their ability to provide a wealth of biological data relevant to other species (most notably humans) that are more difficult to study directly. The nematode *C. elegans*, for example, has an invariant body plan, undergoes multicellular biological processes such as embryogenesis, growth, reproduction, and aging, is small in length, generates rapidly (2–3 days), has a short lifespan (2–3 weeks), and is economical to grow in large numbers.[86] Despite its undoubted utility in biological research, its small size has proved to be a major barrier in allowing large-scale experimentation (a key requirement in many contemporary studies). Indeed, the most common methods for worm manipulation and inspection are manual and based on observations under a microscope, where individual worms are handled with small metal hooks or pickers. Although methods for isolating the large number of adult worms needed for transcriptomic and metabolomic studies have been proposed, such as chemical sterilization and mechanical sorting, each has major drawbacks in terms of complexity, cost, timescale, or perturbation of the animal's biology. To this end, a range of microfluidic tools for handling, processing, and probing *C. elegans* in high throughput and with high resolution are beginning to be developed. For example, microfluidic devices have been used to deliver specific stimuli and record sensory neuronal and behavioral responses in *C. elegans*.[87] In addition, arrays of connected microfluidic traps have been used to automatically isolate and immobilize hundreds of worms for imaging and microsurgery applications.[88] Such a strategy allows screening of phenotypic features at subcellular resolution in physiologically active animals. Other technologies have also demonstrated the suitability of using passive microfluidic tools for sorting worms from mixed populations according to their size and motility; a tool that could eventually allow for simple, high-throughput ($>5 \times 10^5$ worms/hr), and automated worm selection based on phenotype.[89] All of the above approaches leverage the ability to control small entities within low Reynolds number regimes, and are likely to allow the extraction of novel biological information, especially in the fields of genetic screening, aging, and developmental biology.

Susceptibility Screening

Current techniques for antibiotic screening are slow by their very nature. A culture of the pathogen must be grown, and typically a disc trial or PCR-based assay is performed to assess the agent of choice.[90] This approach is so slow that in practice it is rarely done outside of a hospital setting. Typically, a broad-spectrum agent is given to a patient followed by other agents if ineffective. This is a serious problem in that it exposes organisms to a variety of agents, and thus reinforces resistance. Because the rate-determining step in the process is culturing, a culture-free or

low-culture approach would be game changing. Several studies have demonstrated just how microfluidics might be used to create such systems, from the straightforward detection of MRSA organisms[91] through to generalized susceptibility screens, with screen times of less than 3 hr being reported. Continued refinements are expected and likely to provide for sample-to-answer times within minutes, which would allow only the most effective compounds to be prescribed. Similarly in cancer treatment, it is widely recognized that different neoplasms respond to different chemotherapeutic agents. Screening for these susceptibilities via PCR, sequencing, or other genetic techniques is already happening in some oncology centers,[92] and of course microfluidics can accelerate these processes. However, the capture of CTCs will potentially allow for the direct screening of chemotherapeutic agents against the metastatic cells themselves.[93]

Molecular Evolution

As previously noted, the use of microfluidic technology to measure and characterize single-molecule events has been arguably one of the most successful applications of microfluidics in recent years. Single-molecule assays typically require each molecule to be spatially separated into distinct and isolated volumes, after which these volumes are manipulated and analyzed either in parallel or in a high-throughput fashion.[94] Microfluidics excels in its ability to create large arrays of small, isolated, and well-defined volumes (via chambers, droplets, or a combination of each), and indeed the evolution of digital PCR into a successful commercial technique serves to illustrate this capability.[95] Digital PCR, initially also known as "limiting dilution PCR," requires a sample of interest to be divided into an array of volumes such that a small number of volumes contain only one copy (at most) of the DNA molecule of interest. Subsequent PCR results in amplification (normally reported via fluorescence) only in the few volumes that originally contained a single copy of DNA (Figure 6A). Quantification is precisely and simply determined by counting the number of volumes that contained a copy of DNA. Although the concept of digital PCR was first demonstrated with well plates in the early 1990s, its widespread adoption had to wait until microfluidic systems enabled it to become an easy-to-use and robust technique in the everyday lab (Figures 6B and 6C). A similar development cycle is currently taking place in digital ELISA, where large arrays of magnetic beads are housed in enclosed wells. Such structures confine the enzymatically amplified molecules that signal the presence of the analyte. This ability to confine amplified reagent molecules within the immediate volume of a single molecule, such as an enzyme, or a cell that manufactures antibodies, is also finding utility in in vitro evolution and the generation of synthetic molecules[96] and is likely to significantly have an impact on general laboratory practice.

Catalyst development is perhaps not a field where evolutionary terminology is readily used, but in fact the principles of divergence and selection are widely applied. It is quite normal to screen large numbers of candidates in order to find a successful catalyst, and unsurprisingly microfluidic technologies have been applied to this effort for quite some time.[97] The high-throughput nature of microfluidic reactors means that catalysts and operating conditions can be rapidly assessed, and this continues to be applied and developed for more complex systems such as enzymatic processes. Importantly, however, microfluidic systems are beginning to be coupled directly to genetic-algorithm-controlled experimental systems in order to evolve the ideal catalytic system.[98] This form of feedback-driven system is likely to make a major impact in the industrial selection of catalysts and enzymes.

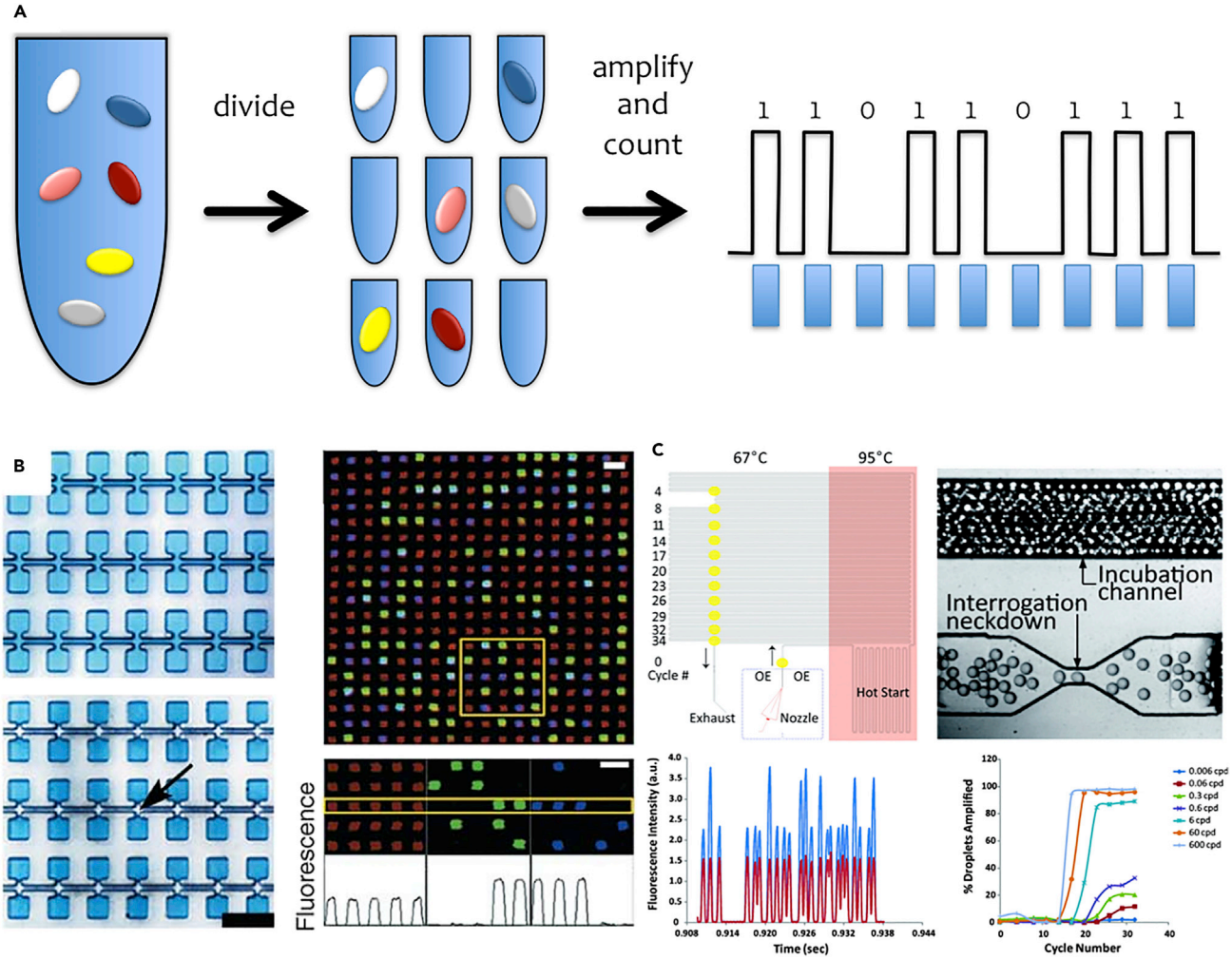

Figure 6. Digital PCR

(A) In digital PCR, a sample is partitioned into many smaller fractions so that each fraction contains either one or no template molecules before PCR (limiting dilution), thereby enriching minority targets within individual reactions. After PCR amplification, nucleic acids are quantified by counting the fractions that contain PCR end product.

(B) Megapixel digital PCR using planar chamber arrays. Optical micrograph of reaction chambers filled with blue dye (top left) and after partitioning with an immiscible fluorinated oil (bottom right). An expanded view of 342 chambers illustrates the detection of specific sequences from human genomic DNA in green and blue (top right). An intensity profile is shown across a highlighted strip of five chambers (bottom right). Reprinted by permission from Macmillan Publishers Ltd: Nature Methods (Heyries et al.[17]), copyright 2011.

(C) qPCR in picoliter droplets. Top left: schematic of the overall flow configuration. Pink regions are maintained at 95°C, and non-shaded regions are maintained at 67°C. Yellow dots correspond to the interrogation neckdowns. Top right: image of droplets in the downstream channel and flowing through a neckdown where they are interrogated. Bottom left: a 36-ms fluorescence time trace showing signals from a control fluorophore and a labeled TaqMan probe specific to a region of an adenovirus sequence. Bottom right: percentage of droplets with fluorescence above the background level (i.e., PCR-positive droplets) plotted against cycle number. Reprinted with permission from Kiss et al.[106] Copyright 2008 American Chemical Society.

Epigenetics

One key area for microfluidic development is single-cell epigenetic analysis, which is currently at the edge of existing technologies. The inherent inefficiencies of existing procedures, coupled with the need to reliably detect single alleles, makes these applications particularly challenging. However, given the rapid advancement of single-cell RNA sequencing, it is likely that low-template protocols will soon be available and that the transfer of these to the microfluidic format will be important to achieving the required sensitivity.[99]

Given the enhancement in nucleic acid concentrations achieved in small volumes, microfluidic processing could very well remove the need for RNA or DNA amplification altogether, allowing direct single-cell library construction using traditional ligation or more recent transposon approaches. Another important trend will be maximizing the throughput of single-cell analysis. Whereas valve-based systems are scalable to several hundred cells per experiment, droplet-based microfluidics would appear to be unmatched in raw throughput, capable of processing millions of reactions per run. When coupled with DNA-indexing strategies, this can prove an exceptional approach for targeted sequencing of antibody and T cell receptor genes. Achieving such aims will require the development of simplified protocols that can work efficiently at high-lysate concentrations; ironically, the most convenient droplet or chamber sizes (between 10 and 100 pL) might actually be too small, resulting in reaction inhibition by cell lysate. This highlights a general truth in microfluidics, namely that technology development involves much more than hardware and must include optimization of assays that are tailored to the microfluidic format of choice.

Materials Science

Microfluidic channels are now being exploited to synthesize complex materials spanning the nano- to millimeter scales. For example, rapid mixing using segmented flows, multistep chemistry, and integrated separation steps make microfluidics a powerful platform for synthesizing a wide variety of nanomaterials, where particle shape, size, and size distributions can be controlled with unrivalled precision.[58] Significantly, microfluidic devices readily allow for in-line monitoring and are naturally continuous (rather than batch), both of which lead to superior process control and thus preferred from an industrial standpoint.[100] Indeed, the production of highly monodisperse droplets[18] has proved an important stepping stone to the synthesis of complex microparticles. For example, droplets produced by two-phase flows can be solidified by chemical or physical crosslinking to form spheroidal particles.[77] Not only are the particles monodisperse, but the controlled co-flow of two fluids, which are then pinched off to form a droplet, can result in Janus or two-sided microparticles. Even more sophisticated microstructures can be formed by forcing droplets into other droplets to make designer double or triple emulsions.[101] Select regions of these fascinating compound particles can then be solidified to create thin shells. Another approach to making highly complex particles is to use co-flows to precisely arrange pre-polymers and then a pulse of masked UV illumination to optically stamp out particles in a semi-continuous flow lithographic process.[77] Various functional barcoded particles have been produced with this technique.[3] Continuous co-flowing of fluids can also give rise to structured fibers and ribbons. Inspired by the spindle of a spider and enabled by programmed flow control, hydrogel fibers containing spatially varying chemistry and embedded objects (e.g., cells) have been formed.[102]

Particle synthesis in microfluidic devices is still a relatively new field, and the future impact of the technique will be tightly coupled with applications. In part as a result of rapid materials screening and consistent batch-to-batch quality, microfluidic tools will be enabling in accelerating clinical translation of nanoparticles. Formulation and delivery of dietary supplements and food is a relatively untapped area that would greatly benefit from structured soft matter (e.g., edible alginate capsules). The motifs of controlled compartments, structured morphology, and biocompatibility are well positioned to have an impact on pharmaceutical manufacturing and tissue engineering.

THE FUTURE

We hope that our brief analysis has to some degree highlighted how microfluidic technologies are currently used in the chemical and biological sciences, where they have been used to good effect and where, in the future, they stand to do the most good. Naturally, such a limited analysis is biased by personal experience and expectation, and as a result we are likely to have omitted comment on many applications and uses of microfluidic tools that will become apparent in the medium to long term. Regardless, a primary intention in writing this article was to provide some opinion on how the field of microfluidics should develop in the forthcoming years.

Although microfluidics can provide the experimentalist with many advantages, it is not a cure-all. Indeed, many features of microfluidic systems that were once deemed critical to their use or adoption are now less compelling to attendant improvements and refinements in conventional technologies. In simple terms, a microfluidic tool must make a persuasive case for adoption on the basis of factors such as analytical performance, usability, and information yield. For example, although the advantages of transferring macroscale electrophoresis platforms to chip-based formats are undeniable (in terms of speed, throughput, integration with downstream processing, and analytical efficiency), many molecular biologists still prefer to pour macroscale gels (because this yields essentially the same information albeit on longer timescales). Conversely, and as previously discussed, the adoption of (droplet- or chamber-based) microfluidic tools for performing DNA amplification has facilitated robust and efficient digital PCR in a manner previously unattainable by conventional technologies. Not surprisingly, uptake and commercialization of such approaches has been almost immediate. Accordingly, a rather obvious but important challenge to the community is to recognize that microfluidic tools are not unequivocally enabling (or appropriate) in many situations and that the ability to identify the right applications, which find immediate benefit when leveraging microfluidic control, will be critical in fostering more widespread adoption of the technology set.

At a more fundamental level, it is clear that the field of microfluidics has undergone significant developments since its formal inception in the early 1990s. Initial publications articulating novel technological innovations with "potential application" are beginning to be supplanted to some extent by studies that illustrate new biological or chemical science that has been directly facilitated by microfluidic control. Such an evolution does not occur overnight and it is fair to say that the largest proportion of publications incorporating "microfluidic components" still have a strong technological rather than application focus. This is perhaps unsurprising because of the "publish or perish" reality of academic research, but it does place an artificial importance on incremental science without providing convincing arguments for why new tools should be used over existing ones.

The automation of microfluidic design potentially provides an intriguing remedy for many of the above issues. Currently, the realization of functional microfluidic systems in most laboratories involves a large degree of handcrafting, hands-on experience, and empirical knowledge. Although this has not prevented innovation, it has meant that many microfluidic systems unnecessarily exhibit suboptimal performance. In this respect, microfluidicists can learn much from electrical engineers in terms of automating design. Indeed, monolithic integrated circuit designers rarely if ever touch a semiconductor wafer or probe station. Consequently, the need for software that abstracts microfluidic components and then combines them in a way that allows them to function predictably is all too clear.

Microfluidic systems have come a long way toward fulfilling many of the early predictions regarding performance, utility, and functionality. However, they are yet to truly realize their potential as enabling tools in chemical and biological experimentation. To aid future endeavors in the field, we finish with two suggestions. First, think through to the end product. Understand how both the basic and applied work you are doing today will have an impact on something people will want to use in 1–5 years, while taking into account the context of other technologies being developed. Second, explore a concept, physical phenomenon, or design paradigm that is so novel that no one knows what to do with it yet. This could potentially have an impact on many fields because no one is aware of it or has thought of it in combination with other things.

For those readers whose expertise lies outside microfluidics, our final thought is that more and more "microfluidic-embedded" instruments will soon be available. However, just like your smartphone, this technology will be seamless, and all of the engineering, chemistry, and physics that went into it will not be transparent; it will just work, and it may very well work with your smartphone.

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