Journal of Micromechanics and Microengineering

TOPICAL REVIEW • **OPEN ACCESS**

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To cite this article: Marie Hébert *et al* 2022 *J. Micromech. Microeng.* **32** 043001

OPEN ACCESS
IOP Publishing
Journal of Micromechanics and Microengineering
J. Micromech. Microeng. **32** (2022) 043001 (15pp)
https://doi.org/10.1088/1361-6439/ac545f

Topical Review

A perspective of active microfluidic platforms as an enabling tool for applications in other fields

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Received 16 November 2021, revised 25 January 2022
Accepted for publication 11 February 2022
Published 1 March 2022

Abstract

Microfluidics has progressed tremendously as a field over the last two decades. Various areas of microfluidics developed in fully-fledged domains of their own such as organ-on-a-chip, digital and paper microfluidics. Nevertheless, the technological advancement of microfluidics as a field has not yet reached end-users for independent use. This is the key objective that is kept as a lens throughout this review. The ultimate goal is for microfluidics to be simply considered as a tool for application-focused research. A modular automated platform is envisioned to provide the stacking and modularity required to lower the knowledge barrier for end-users. The literature considered in this review is limited to active microfluidics and the analysis focuses on the potential for end-users to independently leverage the platforms for research in various fields such as cell assays, biochemistry, materials, and environmental factors monitoring.

Keywords: droplet microfluidics, active control, modular system

(Some figures may appear in colour only in the online journal)

1. Introduction

As a field, microfluidics evolved and progressed over the decades of research supported by contributors from around the globe and from varied backgrounds. The state of the field with respect to its overarching vision is herein considered through the lens of active microfluidic platforms that are used as a tool for applications in other fields. This review aims to focus on pertinent studies from the vast microfluidics literature. Both single and multi-phase microfluidic platforms will be considered, but only liquids will be included.

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1.1. Historical perspective and future direction of the microfluidics field

Although multiple early studies undoubtedly contributed to the emergence of the microfluidics field, most articles trace back the infancy of microfluidics to the 1990s [1, 2]. The initial microfluidics studies leveraged the mature manufacturing processes from the semiconductor industry (i.e. lithography) [3]. The vision was to miniaturize the total analysis system (TAS) into a single miniature device that takes the sample in and provides the answer [1]. The evolution of such innovative vision is still ongoing. As per one of the former presidents of Bell Labs, Mervin Kelly, innovation progresses from fundamental scientific discovery to product development before reaching the market [4]. Many studies established strong fundamentals

both through theoretical [5] and experimental [2] work. The focus of the publications is now increasingly unto product development for various applications. However, the translation from academic studies to market products is challenging; fundraising many millions and spending years are necessary to bring a microfluidic product to market for the end-users [6].

The initial vision of μTAS persists through the more recent microfluidic developments; the field nevertheless expanded into multiple branches with diversified objectives and scopes. The ambitious goal of achieving μTAS involves processing a sample to perform a series of chemical analyses from preparation to detection; a generally more manageable scope is to only consider one or a few steps at a time using a lab-on-a-chip (LOC). Some LOC microfluidic devices are not only developed in academic laboratories [7] but also as commercially viable products. There are numerous companies with products in the market, but due to the cost and operational training required, the benefits of switching from standard protocols to a microfluidic approach do not often outweigh the hurdles [8]. Hence, the impact of microfluidic products in other fields such as chemistry and biology is still somewhat limited. Ideally, biologists and chemists would view microfluidics simply as a tool [9].

The path to achieving the vision of microfluidic as a tool is unclear, but Prof. Whitesides proposed progress similar to that of our modern-day computers [10]. The key feature is stacking; the basic building blocks are reliable, predictable, and repeatable. The analogy of how μTAS relates to the component level aspects relates to how people use websites. The user do not need to understand how the Internet works, or how the different components of their computer interact, or how integrated circuits (ICs) operate, or how transistors constitute the different ICs. The building blocks of each previous level are reliable, predictable, and repeatable enough to allow stacking. Hence, the users do not need thorough electronics understanding to obtain the results of a Google search. Another analogy is how a chemist or a biologist can disregard the inner workings of an electronic balance when they wish to weigh a sample. They simply place their sample and the weight is displayed: sample in, answer out. A similar development in microfluidics would allow application-focused researchers to use these stackable blocks to build systems with new capabilities and fully leverage the potential of microfluidics.

Microfluidics-focused studies typically motivate their device through applications in numerous fields such as water treatment [11], life biology [12], and material synthesis [13, 14]; however, the impact of application-focused studies has mainly been restricted within the microfluidics community. Thus, the field is still searching for pivotal applications that will propel microfluidics from academic development research to end-users' applications. Microfluidic tools have the potential to get at the forefront of impactful discoveries in related fields.

1.2. Scope and motivation of this review

The limited use of microfluidic tools outside of the community motivated this review to help not only identify the progress but also the gaps required for wider adoption of microfluidic devices as tools. Researchers from the biochemistry, pharmacy, material science, and many other fields could benefit the most from the advantages that microfluidic devices present. However, the difficulty in adopting microfluidics simply as a tool without requiring an in-depth understanding of the underlying principles dampens the global impact of microfluidics.

In order to remove the knowledge barrier, users should not be required to understand how the building blocks function as they should be reliable, predictable, and repeatable. In contrast to passive approaches, active methods are promising to achieve this objective. Passive systems are sensitive to manufacturing tolerances and operational uncertainties. Hence, they require a good understanding of fluid mechanics at the micrometre scale to operate robustly. Conversely, active approaches can eliminate the intuitive adjustments required by the user that are based on in-depth knowledge and previous microfluidic experience; adjustments are handled automatically through the active component of the platform.

An important drawback of certain active methods is the low-throughput compared to passive methods. Nonetheless, the contribution of microfluidic devices is more promising as an analytical tool than as a production means; microfluidics more efficiently provides information than products.

The main categories of application of microfluidics will be summarized to provide the reader an understanding of the motivation of end-users and their goal. Then, studies focusing on the development of modular microfluidic platforms–either stacking or modular-focused–are analyzed. The microfluidic active control platforms are then categorized into single-manipulation, single-application or platform technologies. The versatile microfluidic platforms align best with the μTAS vision, but elements from the other studies are required to achieve the vision.

2. Applications in many fields

A few categories of applications are selected to highlight the potential of microfluidics for end-users. The studies involving cells are separated according to their scale (single-cell, multicell, organism) and more fundamental studies. Biochemistry research focuses particularly on the advantage of the small volume required for microfluidic devices. Materials studies typically produce nanoparticles by leveraging the short reaction time of microfluidic technologies. Monitoring environmental factors is achieved for portable microfluidic devices. Furthermore, impactful studies are singled out in a separate section; their impact is not quantified through publishing analytic but rather by providing unique capabilities impossible without microfluidic methods.

The early motivation arising from the TAS vision focuses on obtaining answers from samples through chemical analysis. Microfluidics was applied to many different fields with different objectives. The purpose of each application can be generally classified as either information-focused or production-focused. The small volume involved in microfluidic reactions provides key advantages [15] for reagent consumption and

reaction time to information-focused applications. Nonetheless, production-focused applications provide valuable contributions through efforts of parallelization to increase yield and synthesis of high-value-added compounds.

2.1. Cells

The similar scale of microfluidic devices and single-cells are leveraged in many studies [16–22]. For example, circulating tumour cells (CTCs) are localized in the height direction of a channel using dielectrophoresis electrodes; the CTCs pass through a localized lethal zone that eliminates them without affecting the healthy blood cells [23].

Microfluidic studies also scale up to involve multiple cells. Organs-on-a-chip are a collection of cells mimicking whole organs or organ systems to model *in vitro* processes [24]. For example, a human model of the transport of sodium-coupled glucose of the renal proximal tubule is studied using a microfluidic device to understand the effects of administrating a sodium-transport inhibiting drug [25]. Additionally, the long-term flow through human intestinal organoids is studied using microfluidics [26]. The microfluidic *in vitro* models are not restricted to humans; the fish intestinal barrier [27] are studied using organs-on-a-chip. In contrast to organs, microtumours are another multi-cell environment. Flow conditions enables the control of diffusive and conductive mass transport of anti-cancer drug delivery to perform drug screening studies [28].

On an even larger scale, whole organisms are studied using microfluidic devices. *Caenorhabditis elegans* organisms offer a platform for *in vivo* drug screening that is physiologically relevant to humans [29, 30]. Their adult size of 1.3 mm is larger than the micrometre scale, but microfluidic devices provide added control and throughput to study Parkinson's disease for example [31].

A conspicuous challenge of biology-focus μTAS is the non-Newtonian behaviour of whole blood and other biological fluids with its misunderstood impact for flow in microfluidic devices [32]. Blood is a significant source of information for biological assays and is a key sample to process through μTAS. In addition to its non-Newtonian behaviour, blood coagulation causes issues. Remediation strategies include chemical or pharmaceutical approaches but the depletion of the active components and the potential for interference cause problems. Dielectrophoresis is explored as a potential solution, but there is still no widely accepted and used solution [33]. There are nevertheless promising advances in the microfluidics field that can handle whole blood through the enrichment of extracellular vesicles by using a magnetic bead-based approach [34]. Furthermore, a digital microfluidic platform incorporated a blood-plasma separation membrane; the blood sample is easily obtained from a finger and the device avoids any sample pre-processing by filtering to deal only with the plasma [35]. More fundamental work is necessary to establish a solid understanding of the complex behaviour of non-Newtonian fluids in microchannels. This is especially important for whole blood considering the dependence of its behaviour on its content among other factors.

Studying cells at the unit level is important for diagnostic and personalized medicine. However, the cells are challenging to handle at the individuals level because of their small size. Droplet microfluidics offers a solution by encapsulating the single cells in droplets. Active droplet microfluidics enables a greater control over the droplets. However, approaches that require complex devices such as embedded electrodes and design optimization are cumbersome to use. Nevertheless, the numerous studies showcase the significant impact active microfluidics can have for cell-focused applications. A platform-based approach is envisioned to provide an integrated solution. Nevertheless, further development towards a modular automated platform is required to palliate the knowledge barrier users face.

2.2. Biochemistry

Chemical reactions for biochemistry purposes do not necessarily involve cells; drug screening and protein crystallization are two examples. The microfluidic devices is information-focused. The combination of the different samples and the analysis of their response is the output. Microfluidic devices enable the users to use small quantities of reagents and achieve the reactions faster. Active droplet control proposes to efficiently and systematically investigate the parameter space.

The small sample volume involved in the reaction at the micro scale saves reagent. This advantage is particularly significant for sparse samples such as the cerebrospinal fluid from a mice [36] and expensive samples such as those used for drug screening assays [37]. Multiple droplet manipulations are required for drug screening assays. Droplets containing each solution are generated in parallel, then, they are trapped, merged and monitored in a passive microfluidic device [38, 39]. Although the complex manipulations are achieved passively, translating the microfluidic device to a commercial product or device suitable for end-user independent use is a complex endeavour. The structure of microfluidic also enable a greater control over the drug delivery mechanisms [40].

The rapidity and lower consumption of reagents are advantageous to probe vast parameter spaces. The search for chemical conditions for the crystallization of proteins enables the study of their structure mainly for medicinal purposes. Microfluidic tools enable crystallization of otherwise recalcitrant proteins when subjected to traditional larger-scale approaches [41–44].

2.3. Materials

Complex emulsions and nanoparticle synthesis are achieved using microfluidic devices. The capabilities of manipulating fluid at such a small scale with better uniformity than bulk methods are promising [45–51]; however, achieving significant yield with microfluidic devices is challenging. Thus, most applications focus on using microfluidics as an analytical tool to provide information rather than to yield a product. Nevertheless, microfluidics is leveraged to achieve a narrower size distribution through better control of nanoprecipitation

using an acoustically-driven micromixer for example [52]. As for complex emulsions, microfluidic devices allow a higher level of control over the number, size, and type of internal droplets that is not achievable with non-microfluidic approaches [53–55].

Similarly to the biochemistry applications, material-focused applications benefit from the short reaction time of droplet microfluidics. Moreover, the flow manipulation capabilities and control enables better uniformity and complex structures. Active droplet microfluidics has the potential to automate the scanning of vast parameter spaces. An automated modular platform is required to enable end users without in-depth microfluidic knowledge to leverage these advantages. Current active approaches often require complex fabrication and optimization.

2.4. Environmental factors monitoring

The application of microfluidic tools to monitor environmental factors is focused on gathering information that is critical for safety. Various approaches involve microfluidic tools for their small sample volume required, fast response, and low cost. The small size of microfluidic platforms enables portability and point-of-care (POC) decentralized testing. Selectivity is important to target the desired compound within the complex chemical profile of water samples. Sensitivity is essential to detect low concentrations of harmful chemicals that are detrimental to health and the environment.

The distinct approaches developed have different requirements and performance. Mercury and arsenic are among the species of interest that are detected. Mercury ions are sensed with concentrations from 2 to 12 mM and only require a 2.8 μl sample [56]. Another electromechanical method enables the detection of arsenic in the range of 1–150 $\mu g\,l^{-1}$ concentration [57]. Although another study requires a larger sample of 40 μl, its operating principle does not involve valves and is thus compact and portable [58]. A simple approach that does not require moving parts is paper-based microfluidics that is used to target copper ions for example [59]. Specificity is challenged for certain platforms. Detecting different ions is impactful and is achieved visually for mercury, lead and copper using DNA-nanoparticle probes; the detection limit for all three ions is on the order of 1 nM [60]. Furthermore, certain micro-organisms present in water are also detrimental similarly to heavy metal ions, and the detection of the micro-organisms is thus also important [61].

Focused review articles provide a targeted point of view about environmental factor monitoring. The combination of microfluidics with a smartphone is particularly potent for portable solutions [62]; furthermore, image-based colourimetric sensing techniques are ideal for portability and low cost [63]. As for electro-mechanical techniques, nanomaterial-based modification of the electrodes increases the performance through enhanced specificity and sensitivity for different metal ions [64, 65]. Finally, samples are targeted at various points in the water cycle such as wastewater monitoring with biosensors [66] and various approaches to detect metal cations in drinking water [67].

2.5. Impactful applications

The most impactful applications of microfluidic devices are the ones providing key advantages and possibilities compared to bulk methods. The high-throughput, low reagent consumption, short reaction times, and reaction compartmentalization are interesting advantages. However, they do not always justify the switch from a more familiar method to microfluidics. Microfluidic devices are more easily adopted when providing new capabilities or definitive advantages [68, 69].

The laminar nature of flow at the micron scale is challenging to thoroughly mix the sample. Nonetheless, it is also leveraged to control the diffusion. Hence, using a simple approach with a syringe pump, denaturalized proteins and the buffer are passed through a chip side by side. The resulting refolding of the proteins is more effective and does not require a post-processing purification step comparatively to other non-microfluidic-based approaches [70].

The strength of microfluidics more commonly lies in the information it provides rather than the yield it produces. High-value products such as drugs can nevertheless be pertinent applications. Parallelization of micro-reactors yields higher throughput by increasing the production 25 fold to achieve 31 $g\,h^{-1}$ [71]. The miniaturization and implementation of the instrumentation on the microfluidic device enables *in-situ* measurement. These are particularly useful for transient measurements. For example, a by-product of the oil industry is deposited on porous media and its temperature properties are examined [72]. Microfluidics enable an extensive control over the arrangement and the complexity of the microstructure. Researchers leverage these capabilities and small scale of microfluidic tools to work towards the generation of artificial cells [73]. Micro-droplets provide a platform with better frequency control of Belousov–Zhabotinsky reactions. Thus, further insights enable a better understanding of the phenomenon [74].

These examples demonstrate the significant potential impact of microfluidic in application-focused fields. However, passive approaches lack the control ability to enable ease of use by end-users. Active droplet microfluidics enhances the control of droplets; however, the complex manufacturing with integrated electrodes for example is prohibitive to the widespread adoption. Instead, an approach with simple fabrication and hardware should be prioritized. The control handles the microfluidic aspects such that people without extensive in microfluidics and controls can leverage the platform. Moreover, the active microfluidic platform should be modular to enable the end users to easily adapt it to fulfill their needs.

3. Modular platforms

3.1. Framework overview

The development of microfluidic tools that are easy to be used in other fields requires reliable, predictable, and repeatable building blocks as formulated by Prof. Whitesides [10]. The stacking principle enables the usage of higher-level functionalities without requiring an understanding of the lower

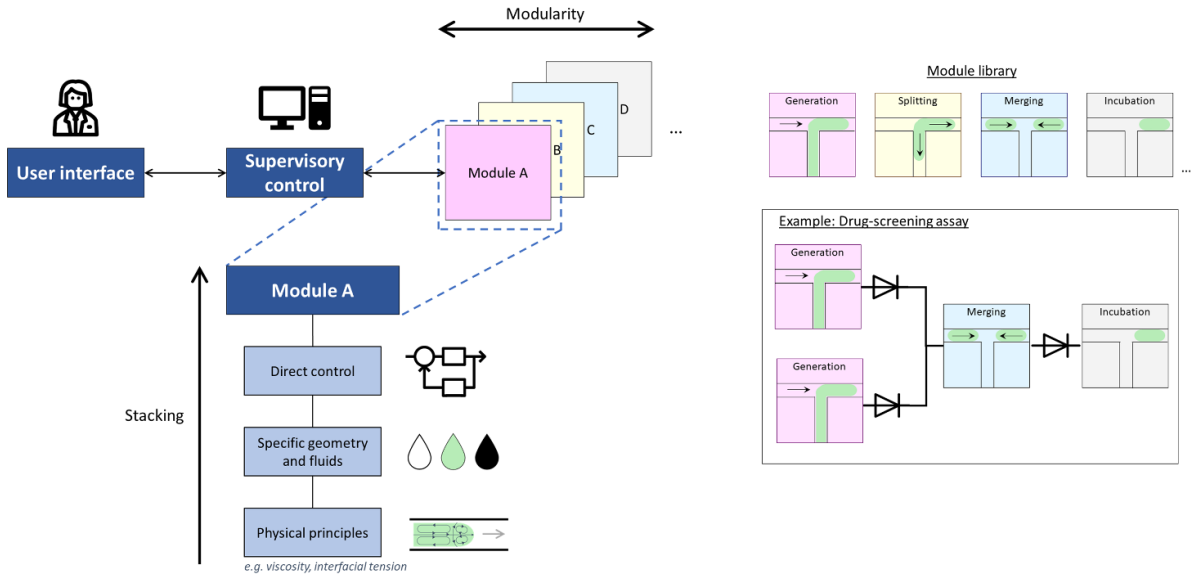

Figure 1. Stackable modular platform structure and vision for easy control of individual droplets by end-users. Structure of a stackable modular platform. The vertical direction shows the stacking while the horizontal direction shows modularity. Each manipulation is a module that leverages stacking such that only high-level information from the user is required.

level building blocks. For microfluidic devices more specifically, stacking signifies that end-users perform manipulations without requiring an in-depth understanding of fluid mechanics at the micro-scale. As illustrated in figure 1, vertically, the building blocks must exhibit reliability, predictability, and repeatability–that is stackability–for the users to depend on them. Horizontally, the modules must operate independently to avoid influencing each other, but appropriate exchange channels must be established between them. In the envisioned modular system, each module is responsible for one droplet manipulation that corresponds to one step of the assay. The operation of the modules is controlled by the supervisory control layer that interprets the user input as instructions for each module. Moreover, progress in the natural language processing field has the potential to simplify user input. A potentially cumbersome user interface is replaced by the direct interpretation of a user's directives [75].

The value of modular design has already been leveraged in several studies. However, generally, the lack of either independence or stackability of the modules compromises the potential of the platform. Thus, the literature covers various aspects required to achieve an impactful modular platform but separately.

3.2. Modules

Certain studies focus on developing modules that the user assembles for their specific use; the approach involves either single-phase or droplet flow. Single-phase flow modules behave more independently from each other than droplet flow modules; the dynamic effects of droplets travelling through channels that are coupled through the pressure field easily compromise the independence of each module [76].

Therefore, influence between the modules is circumvented by using single-phase flow for an automated modular approach [77, 78]. However, droplet microfluidics provides key advantages such as compartmentalization that are essential to many applications. A critical issue that modular microfluidic systems must address is the connection of the different modules. The focus on the mechanical assembly of the different modules is essential but usually fails to address the issue of dependability [79–86]. The coupling of adjacent modules through the pressure field can result in undesirable dynamics compromising the operation of the device. Nevertheless, a reliable connection between the modules is primordial to the success of modular systems. The component with the flow-driving capabilities is a prime candidate for modularity [87]. However, without applying the principles to the rest of the microfluidic system, the independent usage by end-users is still compromised. Similarly, a heat-exchange module embodies the modularity principle but is limited to the component rather than the whole microfluidic system [88]. The handling of single-cells is automated using a vision-based system. However, the cells are transferred to passive microfluidic channels, and thus, the system is modular and adheres to the stacking principle only for the cell-handling module [89].

3.3. Stacking components

The studies proposing the stacking of components either focus on peripheral components to the chip or the chip itself.

The modularity of the peripheral equipment to the microfluidic chip is essential to the versatility of the whole system; the stacking of the microfluidic chip function is deemed a critical factor that should also be addressed, but that usually is not. For example, an SMR-focused study demonstrates the potential of modular equipment, but the application as a general microfluidic platform is limited [84]. Similarly, modularity is also demonstrated but is limited to the actuation [90].

The devices that allow the sample as the input and provides the answer as the output embody the principle of stacking. The user of the device does not need to thoroughly understand how the device works. However, the devices are sometimes overly tailored to specific applications, for example, detection and quantification of infectious pathogens using dRPA [91].

4. Active control platforms

The historical perspective and key applications of microfluidic devices motivate the vision and need for an automated active microfluidic platform that is used as a tool. A sub-field of microfluidics that concentrates on achieving such objectives are active control platforms. Diverse avenues to achieve the envisioned μTAS are herein included.

The two main objectives achieved are: (a) the integration of chemical and physical sensors for continuous monitoring, and (b) logic and feedback algorithms for automated screening, process control, and optimization [92]. The challenges identified by McMullen and Jensen extend beyond the scope of their article that is focused on analytical chemistry but are nevertheless pertinent to this day.

- Translating the thinking and decision-making skills of a researcher into an automated tool.
- Detecting failures such as clogging to avoid introducing erroneous data into the results.
- Handling the complications that compound as the microchannel network grows.
- Developing algorithms that provide stability, and fast dynamics.
- Collaborating between multiple experts in chemistry, engineering, micro-fabrication, and software development.

The integration of chemical and physical sensors are crucial to monitor the parameters of interest. The two main categories of studies are: (a) screening a vast parameter space for various reactions [93–98], and (b) real-time optimization and change through online feedback [99, 100]. Implementing sensors for the varied parameters that are monitored on the microfluidic chip is complex and focused review articles provide more details [65, 101, 102]. This review will concentrate on the logic and feedback for process control. The active control is categorized into single-manipulation, single-application, and platform studies. Figure 2 provides an overview of the paper structure.

4.1. Single-manipulation focus for droplets

The automation of droplet manipulations is achieved using various methods typically introducing an external force. The common connection between the studies is their objective to automate one single manipulation of droplets. Examples of target manipulations are generation, merging, mixing and sorting. The single manipulation focus signifies that these studies are not platform technologies that would be promising for the μTAS system vision. The main inhibition in developing a platform from these techniques is the localized effect that cannot easily be extended or multiplexed for multiple manipulations. Nonetheless, the variety of external forces used to control the droplet shows the assortment of approaches.

Although an external force is introduced, and thus, the method is active, the studies focusing on a single-manipulation typically layer the external force on top of passive methods. Most commonly, a passive generation of droplets is the first step of these active single-manipulations. Thus, although the active control enables stacking to some extend, the dependence of the approach on passive microfluidic severely inhibits modularity potential.

4.1.1. Generation. Certain review articles focus specifically on the different external forces introduced to actively control the droplet generation process [103]. The introduction of a force such as an alternative current using on-chip electrodes enables the tuning of the droplet size. The generated droplets contain a sodium chloride solution. Moreover, the study demonstrated a negligible difference in the actively controlled generation process using a non-Newtonian fluid, more specifically, xanthan gum [104]. An external magnetic force enables the break up of droplets. The ferrofluid droplets are generated at a junction [105, 106] or the droplets are split up to generate two daughter droplets [107].

An alternative to introducing an additional external force is to adjust the flow-driving mechanism based on feedback. An approach only requiring the typical equipment without any additional components uses the pressure pump or syringe pump as the force, and a camera to acquire the appropriate feedback. Thus, parameters (e.g length-to-width ratio) are tuned using a controller (e.g. PID controller) to adjust the flow rate of the syringe pump to achieve the desired outcome [108, 109].

Droplet-on-demand implements active methods without the high-throughput and lack of modularity of layering the external force on top of passive droplet generation [110–115]. Although the droplets are generated one at a time, they typically enter a passive network. Thus, the control is increased over single droplets rather than multiple droplets. However, the lack of control after their creation compromises the modularity potential of this approach similar to the active droplet generation techniques that are layered on top of passive generation.

4.1.2. Merging and mixing. Microfluidic applications involving multiple on-chip reagents require merging and mixing. Review articles provide an overview of the different approaches as well as techniques to achieve merging [116] and mixing [117, 118] passively and actively. For example, an on-chip microwave sensor enables the selective heating of the aqueous droplets; the non-uniform heating pattern causes effective mixing between the two droplet halves [119]. A mixing module is integrated with Tesla valves on-chip to thoroughly mix samples; the concentration is digitally controlled [120]. The intermittent pulse-width-modulated signal is leveraged to control the concentration of up to six different reagents using multi-layer valves [121]. Moreover, complex flow profiles are achieved, real-time control is enabled for

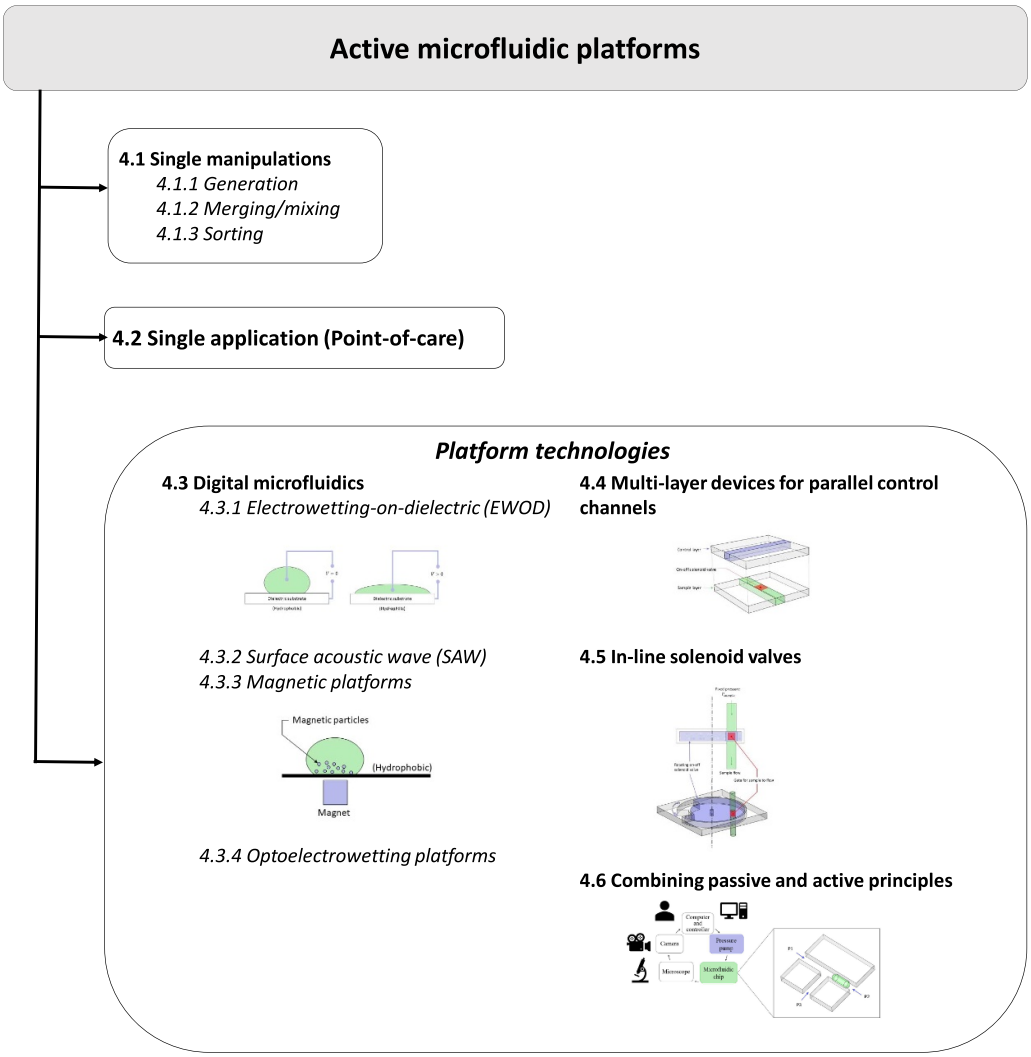

Figure 2. Overview of the active control platforms presented in the subsequent sections.

potential feedback mechanisms, and the necessary design files are provided to replicate the apparatus. Another approach uses magnetic forces to deform an elastic membrane, and hence, improve mixing [122].

4.1.3. Sorting. Sorting droplets inherently requires an active component to deviate the droplets between at least two paths. Moreover, a sensing method must be implemented to detect which droplet to deviate. The need for sorting emerged from leveraging high-throughput encapsulation using microfluidic devices with heterogeneous cell populations. Fluorescence-activated cell sorters (FACSs) is an established tool in the biochemistry field [123, 124]; the fluorescent probes enables the sorting of the cells according to targeted characteristics. The feedback system is generally vision-based such as for fluorescent signals of FACS systems. The study object is not restricted to cells and can be organisms such as *C. Elegans* [125]. More complex identification techniques leverage deep learning to identify the element to actively sort using a pulse [126–128]. Alternatively, the sorting elements span a wider area of unrestricted flow controlled through dielectrophoresis for example [129]. Dielectrophoresis is also leveraged to form a sequentially addressable dielectrophoretic array to achieve large-droplet sorting [130].

Photo-actuation is prominent in sorting manipulations due to its extensive sensing capabilities [131, 132]. Nevertheless, more manipulations such as generation [133] and trapping [134] are achieved. A more detailed review [135] delves into the fundamentals of photo-actuation and the technological developments within the microfluidic community. Another external actuation that enables droplet sorting is a magnetic field. The droplet content is functionalized using superparamagnetic magnetite (Fe_3O_4) nanoparticles for example [136]. The usage of the ferrofluid as the continuous phase rather than as the droplet enables sorting [137] in addition to the trapping of droplets [138].

4.2. Single application: POC platforms

POC platforms are prime examples of the potential impact of microfluidics in other fields, often linked to medical applications. Multiple functions are integrated on a microfluidic device to achieve a specific targeted application [139].

However, the developed microfluidic solutions are highly specific to the target application and lack versatility. Consequently, the end-users cannot adapt the platform to their various need without prior microfluidic knowledge to adapt the design. Nevertheless, the application focus they fulfill demonstrates the potential of microfluidic platforms as tools in other fields.

Paper-based devices are particularly suitable for POC due to their simple operation, compactness, and low-cost. Although the core of paper-based microfluidics relies on passive flow [140], certain active approaches are developed such as thermally activated gates [141].

More generally, POC devices cover a wide range of applications. For example, HIV is detected from whole blood samples [142]. Microfluidic tools are developed and used for iron deficiency anemia [143] and other diagnostics [144–146]. The detection of micro-organisms is also achieved using microfluidic approaches [147].

The great advantage of POC microfluidic devices is their ability to take the sample as the input, and then, provide the answer as the output [148]. The stacking principle is leveraged such that the user does not need to understand the inner workings of the microfluidic device. However, the researchers developing such devices are certainly required to understand microfluidics.

4.3. Digital microfluidics

Digital microfluidics involves larger droplets than those for 'droplet microfluidics'; the difference in scale is one of the major differentiating factors. Oppositely to droplet microfluidics for which the droplets are enclosed in microchannels, digital microfluidics manipulates the droplets on an open array [149]. A widespread approach is electrowetting-on-dielectric (EWOD); surface acoustic waves (SAWs), magnetic forces, and optoelectrowetting are alternative methods. The focus of this section is on digital microfluidics for its ability perform many manipulations and its potential for automation. Therefore, studies focusing on layering the active method in parallel to a passive channel network are omitted. Such studies are covered in the section 4.1 single-manipulation focus for droplets.

4.3.1. Electrowetting-on-dielectric platforms. For EWOD platforms, the droplet size is dictated by the size of the electrodes forming the array upon which the droplets travel. Voltage is applied to convert the substrate from hydrophobic to hydrophilic as schematically illustrated in figure 3. More details about the working principles are included in the relevant literature [150].

The potential for automation and great versatility of the platform led to many applications regrouped in pertinent review articles [151, 152]. For example, a commercially available digital microfluidic device is used to efficiently prepare samples for liquid chromatography-mass spectroscopy [153]. Previously, sample losses and manual manipulations challenged the measurement of low cell number samples.

The high automation and modularity potential are key advantages of EWOD platforms. Multiple companies developed products for main application areas: liquid lenses, reflective displays, and biomedical assays [154]. The potential of the platform is demonstrated for sample preparation. A procedure requiring 4–5 h of manual labour is achieved in about 30 min using an automated EWOD platform. Nonetheless, typical drawbacks of digital microfluidics include sample evaporation, degradation of the samples from the electric field, and detrimental large droplet volume for single-cell analysis. Moreover, non-adherent surface coating properties are essential when using particularly sticky components such as proteins that easily cross-contaminate. In brief, the droplet microfluidic technology is promising in terms of its modularity and automation potential, but inherent drawbacks inhibit its independent use by end-users. The continued development of commercial platforms is nevertheless promising. Any reliability issues must however be first resolved.

4.3.2. SAW platforms. Similarly to EWOD, SAW platforms leverage embedded electrodes (i.e. inter-digital transducers (IDTs)). Review articles explore in more details the fundamentals and capabilities of SAW [155]. The electrode design is generally tailored to the objective of the platform or application; nevertheless, more universal and versatile designs have been investigated [156]. The generation of surface waves using the IDTs requires an electric signal with the appropriate frequency; then, the waves with an amplitude of a few nanometers produce macroscopic effects such as droplet transport [157].

Simple droplet transport is essential and valuable. SAWs are furthermore capable of location feedback in addition to droplet transport [158, 159]. Moreover, multiple droplets are transported independently [160]. The enabling potential of SAW-based platforms arise from the implementation of multiple droplet manipulations. The mixing and sensing capabilities are combined for biosensing of sub-nanomolar concentrations of analytes [161]. Direct heating of droplets is also achieved [162]. The SAW-based droplet transport is leveraged for a PCR application [163].

Furthermore, the combination of SAW with EWOD leverages the strength of each; EWOD is used for transport while SAW mixes the droplet content [164]. Although SAW-based platforms showcase good potential for automation and independent use, they are inhibited by similar disadvantages than EWOD platforms: the fabrication process is complex, the droplets are relatively large, and the platform is susceptible to evaporation.

4.3.3. Magnetic platforms. Magnetic particles contained in droplets enable using permanent magnets or electromagnets for droplet control [165]. The magnetic particles contained in the droplet can serve two purposes: actuation to interact with the magnet, and as a solid substrate for biochemical reactions. The fundamental operating principle is schematically illustrated in figure 4 and is reviewed in details elsewhere [166]. Essentially, the magnet speed and particle concentration determines whether the magnet disengages, the particles

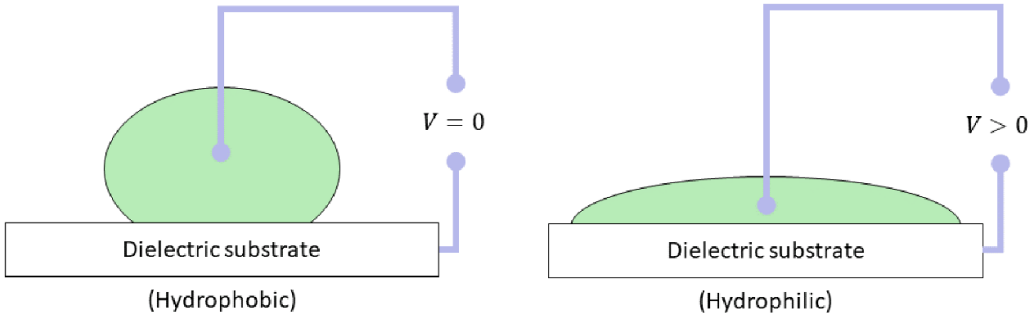

Figure 3. Working principle of EWOD platforms [150]. The applied voltage modifies the surface properties from hydrophobic to hydrophilic. The voltage is applied and independently controlled for a grid.

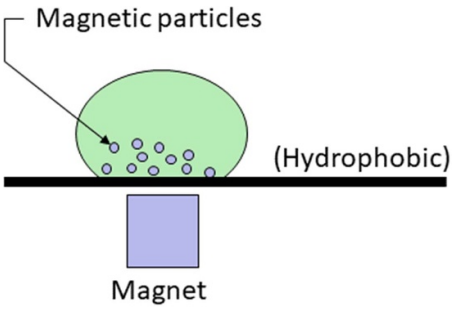

Figure 4. Working principle of magnetic devices with a sample on top of a hydrophobic surface [166].

are extracted or the droplet is steadily transported. The different regimes occur based on the balance between the magnetic, friction and capillary-induced drag forces. The major variables are the particle type, droplet size, surrounding oil layer, surface tension and viscosity.

The magnetic digital microfluidics approach enables droplet generation, transport, merging, and particle extraction [167]. Moreover, surface energy traps enable greater versatility by creating stationary reaction areas [168]. The combination of the magnetic digital microfluidic platform with a heating source demonstrated the potential for genetic detection using PCR. The magnetic beads are particularly powerful as solid substrates for ELISA assays [169–173]; magnetic forces helps extract the bead while the standard EWOD platform handles fluid transport for example [174–176]. A similar approach combining the magnetic bead and forces with the EWOD droplet transport uses a surface enhanced Raman scattering-based immunoassay [177] and the optimization of thyroid-stimulating hormone immunoassays [178].

Similarly to the EWOD platform, magnetic-based digital microfluidics platform provide great automation capabilities. Moreover, the addition of a solid substrate (i.e. magnetic beads) enables many assays such as ELISA and surface enhanced Raman scattering-based immunoassays. However, the same drawbacks than for EWOD affect the magnetic platforms. The droplets are relatively large and susceptible to evaporation, especially when heated for PCR. Moreover, the hydrophobic surface properties is primordial to the operation of the platform and cannot be compromised.

4.3.4. Optoelectrowetting platforms. Optoelectrowetting leverages a similar approach to EWOD. However, instead of applying a voltage to change the surface properties, a light is shined unto photoconductors. The resulting change in surface properties enable a droplet velocity of 7 $\mathrm{mm\,s^{-1}}$ using a 4 mW laster for example [179]. A review provides a wider perspective of the fundamental principles and its usage in a microfluidic context [135].

Many droplets are handled using a 96-droplet array with optimized input and output parameters [180]. Moreover, the surface of the opto-responsive material is modified with nanoparticles for better performance [181, 182]. The actuation is required to be portable and low cost to allow for POC applications. Commercially available smartphones provide an accessible light source. Smartphone-based optoelectrowetting microfluidic platforms with hydrophobic traps enable droplet transport, merging, mixing, detection, and splitting [183–185].

Optoelectrowetting plaforms provide potential for automation and portability using a smartphone-based approach. However, key disadvantages are prohibitive to a widespread use: relatively large droplets and susceptibility to evaporation. Nonetheless, the large droplet size issue is mitigate at the expense of simplicity using a combination of EWOD, magnetic beads and optical tweezers to study single particles and single cells [186].

4.4. Multi-layer devices for parallel control channels

4.4.1. Working principle. The micro-channel network containing the samples are enclosed within one layer; there is at least one other layer that is used for control purposes. On-off valves commanded by a computer provide control over the sample flow within the micro-channel network. Figure 5 schematically illustrates the working principle [187].

4.4.2. Quake's valves. The pioneer of this novel approach–often accordingly labelled as 'Quake's valves'–developed the idea in the early 2000s when microfluidics was still in its infancy [187]. The soft nature of polydimethylsiloxane (PDMS) that has been commonly used for rapid prototyping of microfluidic devices is leveraged to create on-chip valves.

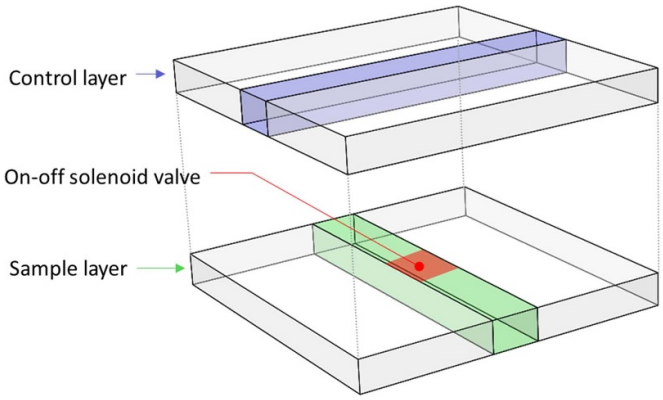

Figure 5. Working principle of multi-layer devices with a sample and a control layer [187]. The zone at the intersection between the two layers acts as an on-chip solenoid valve.

A thin membrane between the sample and control channel is expanded to block flow in the channel using pressurized air. The valves create micro-reactors containing the samples similarly to droplets, although the approach fundamentally operates using single-phase flow.

High-throughput applications require numerous on-chip valves to isolate and to control each micro-reactor. A multiplexing technique reduces the number of off-chip solenoid valves to lessen the burden of the external hardware [188]. Although the microfluidic device footprint is small, the required supporting hardware is substantial. Another drawback is the required more complex fabrication of multi-layer devices that is more demanding than simpler single-layer chips. Finally, single-phase flow is susceptible to cross-contamination; accordingly, special care must be taken to avoid undermining the results. Even considering the aforementioned drawbacks, the Quake's valve platform and similar devices with an air control layer have been applied to numerous studies focusing on applications. They are too numerous to comprehensively list here [189–198].

Although end-users cannot purchase an off-the-shelf commercial product, numerous journal articles detail the process. The multi-layer microfluidic chip process is detailed both for a regular procedure [199] and a 3D-printer-based procedure [200]. Moreover, the supporting hardware required to operate the multiple solenoid valves is accessible through an open-source project [201].

The multi-layer platform is promising due to its demonstrated capabilities with numerous applications and the overall high impact of this research direction within the microfluidic community. Nevertheless, the independent use of the platform by end-users is still not widespread due to the challenges in using the platform and the knowledge barrier; the numerous articles about the process help lower the barrier, but the stacking capabilities are not yet developed enough to the level of a commercial product. The air control layer approach nonetheless occupies an important and promising direction of active microfluidic platforms.

4.4.3. Other approaches. Quake's valves specifically leverage the softness of PDMS to deform and block the sample channels using pressurized air. Similar approaches with the parallel multi-layer control layer relying on different principles have been investigated to integrate valves in microfluidic devices. An electroactive polymer-based valve is triggered by applying a 50 V μm^{-1} electric field [202]. The valve provides enough displacement to close the sample channel and is compact. However, the pressure is limited up to 4.0 kPa (40 mbar). The response time is about 0.7 s.

Alternatively, certain valves are pH activated [203]. The power consumption is low and the thermal risk for the bio-samples is avoided. The valves are hydrogel-based and respond to specific pH solutions to expand and close the channel. The response time is about 10 s. The pH-enabled hydrogel valve footprint is about 500 μm.

Wax valves are melted using heat applied at the desired time [204]. The valves are integrated on a centrifugal disk platform to allow better control of the flow and introduce an active control component. The deformable polymer valves are also integrated within a centrifugal disk platform to control the capillary flow using on-off solenoid valves to an external pressure source [205].

Liquid metal can also be used for electrostatic valves [206]. Additionally, liquid metal can be leveraged in a variety of ways for microfluidic devices [207].

The common objective of adding valves as another layer to microfluidic devices is to supplement the microflow with an active control component. Active control inherently has the potential for stacking of the components. However, a more targeted design solution would enable end-users to more easily and independently leverage the powerful tool that multi-layer microfluidic devices present.

4.5. In-line solenoid valves

Similarly to Quake's valve approach, Garstecki's research group developed an active control method using solenoid valves, but in-line as opposed to in parallel [208]. The valves are directly integrated in series with the flow source as shown in figure 6; hence, the complex multi-layer microchip fabrication is avoided. Furthermore, the microflow involved is two-phase flow (i.e. droplet microfluidics) instead of single-phase flow.

The control capabilities are greatly increased without sacrificing manufacturing simplicity and robustness. Moreover, droplet microfluidics has many advantages over single-phase microfluidics. The reactions are compartmentalized and thus, largely minimize cross-contamination. Multi-phase flow enables high-throughput without compromising uniformity. However, the in-line solenoid valve system still requires involved user interaction to operate the system, and microfluidics knowledge to setup and operate the actuation pressure behind each of the solenoid valve [209]. A combination of this platform with passive traps allowed for more robust operation without knowledge [210]. Nonetheless, the possible

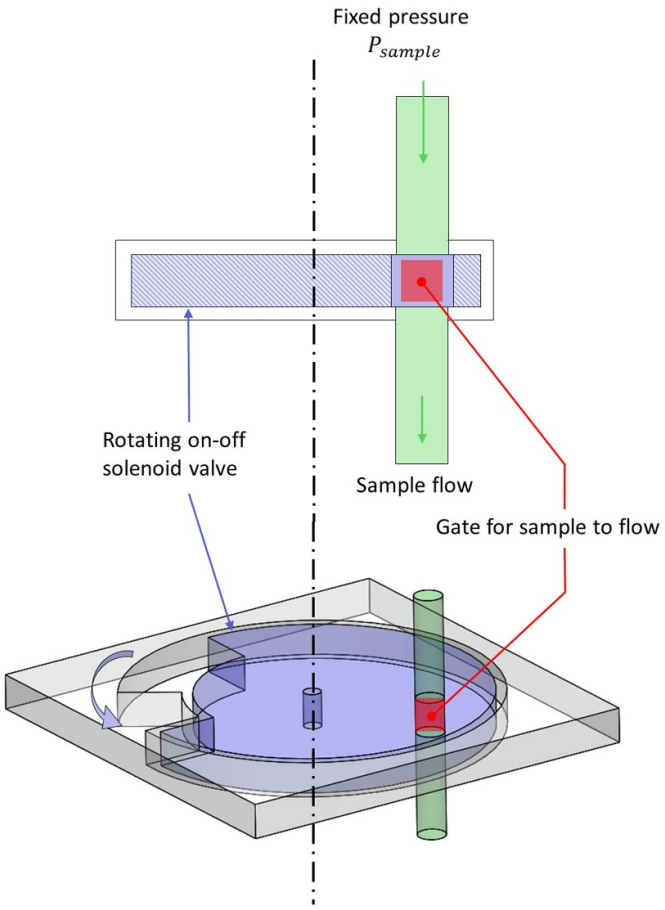

Figure 6. Working principle of in-line solenoid valve [208]. The rotating solenoid valve aligns and blocks the gate pathway for the sample to flow.

manipulations were limited and required more complex microfluidic chip fabrication.

Overall, the in-line solenoid valve offers added control without extensively complicating the manufacturing process. Thus, modularity potential is high. However, the current control approach heavily relies on knowledge of microfluidics and consequently, would require further development to achieve significant stacking capabilities.

4.6. Combining passive and active principles

The control of individual droplets is achieved without introducing an additional force or hardware; the pressure actuation is adjusted based on visual feedback [211–214]. The controller enables the droplets to be manipulated similarly to passive approaches but a much greater control directly associated with the varying control pressure. Thus, this method combines principles both from passive–the use of the geometry to achieve manipulations–and active–varying forces–methods to control *individual* droplets.

The working principle of the active droplet control platform is illustrated in figure 7. The computer hosts the controller that is at the core of the control of the individual droplets. The communication with the other components of the system is also established through the computer. The connected pressure pump individually applies pressure to the inlets of the microfluidic chip. The image analysis is also hosted on the computer to provide the feedback to the controller in the form of droplet location.

A similar approach that uses visual feedback to locate flow has been implemented in the past [215]. However, the controlled localization is not of a droplet, but of an interface. Thus, the potential for applications is much less significant. Another similar approach that incorporates visual feedback and droplet microfluidics is closer to the platform schematically illustrated in figure 7 from Wong *et al*. However, the control is over multiple droplets as opposed to single droplets [109]. Consequently, the rest of the microfluidic device relies on passive droplet manipulations that cannot easily be used by end-users.

The simple manufacturing processes and standard microfluidic equipment required for this platform enhances its potential for independent use by end-users. Moreover, stacking is enabled by the controller which removes the microflow knowledge from end-users. Modularity is not addressed yet but is envisioned to have high potential because of the more simple components required.

4.7. Future outlook

The impact of microfluidic devices depends on their usage by end-users. The developments that are envisioned to be impactful target the knowledge barrier and the financial cost.

The controller at the core of the active platform should balance simplicity and performance. A simpler approach promotes better accessibility for end-users without significantly sacrificing performance.

Commercial pressure pumps provide a turnkey solution. However, their cost can be prohibitive and limit their accessibility to some researchers. A simple approach using a common balloon provides an uncomplicated solution [216, 217]. For more complex requirements, an open-source pressure system provides more flexibility through customization of the different parts. Moreover, the lower cost enables greater accessibility. A minimum understanding of the system is required to properly assemble and maintain the system. Open-source approaches such as μPump [218] provide guidance to lessen the burden of a custom system and reduces cost.

The reliance of microfluidic platforms on microscopes and cameras poses a challenge both for accessibility and versatility. The acquisition of high-performance visualization equipment can be expensive. Simpler and less expensive imaging solutions are important to develop systems that are less costly. Moreover, the dependence of microfluidic platforms on bulky systems such as microscopes limit the portability of the devices.

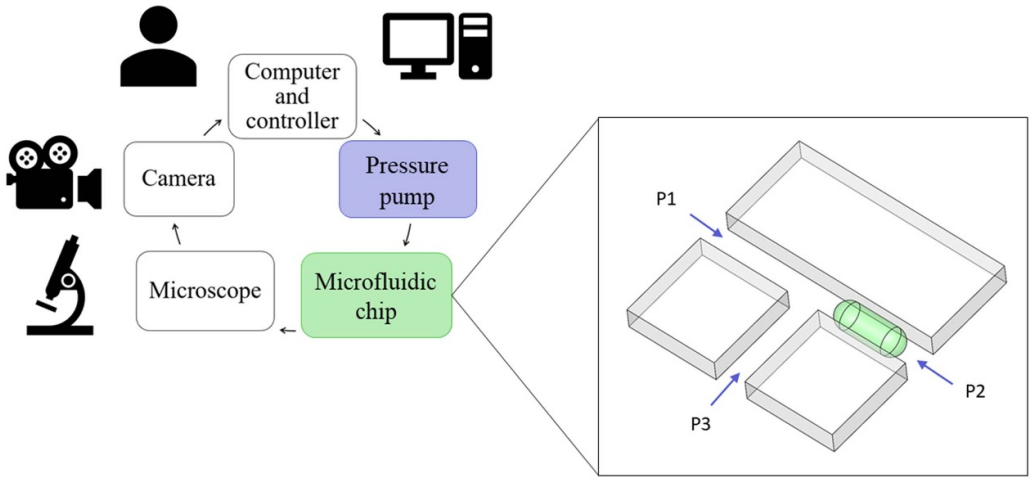

Figure 7. Working principle of the combined active and passive platform [211]. The controller calculates the required pressure to apply at each microfluidic chip inlet based on the feedback provided by the microscope, camera, and on-line image processing.

5. Summary

Microfluidics has tremendous potential to miniaturize TASs (μTAS) for impactful applications. Applications in the fields of cell studies, biochemistry, materials, and environmental factors monitoring motivated the development of numerous microfluidic platforms. However, the adoption of microfluidic tools outside of the developers' field by end-users is limited. A modular platform is envisioned to enable end-users to consider microfluidic as a tool to achieve their goal. Active control removes the microfluidic flow knowledge required of the user. Thus, the knowledge barrier is lower by the stacking of reliable, predictable, and repeatable components.

Certain microfluidic platforms demonstrate stacking capabilities while others focus more on modularity. However, both are needed to achieve a platform that can be used independently by end-users. Similarly, many different active approaches to microfluidics enable the user to by-pass the barrier knowledge typically required by passive microfluidic devices. Therefore, many elements of the envisioned platform are present in various studies. However, they need to be regrouped and optimized in one platform that end users can independently leverage in studies in the various fields benefiting from the advantages provided by microfluidics.

Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

Acknowledgments

This work was carried out thanks to grants from Natural Science Engineering Research Council Canada provided to Prof. Carolyn Ren (RGPIN-2018-04151). Other funding includes NSERC CGS-D scholarship.

Author contributions

Marie Hébert contributed to the writing and editing of the manuscript. Jan Huissoon and Carolyn L Ren contributed to the editing of the manuscript.

Conflict of interest

There are no conflicts to declare.

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References

[1] Manz A, Graber N and Widmer H M 1990 *Sens. Actuators* B **1** 244–8
[2] Kenis P J, Ismagilov R F and Whitesides G M 1999 *Science* **285** 83–85
[3] Verpoorte E, Manz A, Lüdi H, Bruno A, Maystre F, Krattiger B, Widmer H, Van der Schoot B and De Rooij N 1992 *Sens. Actuators* B **6** 66–70
[4] Gertner J 2012 *The Idea Factory: Bell Labs and the Great Age of American Innovation* (New York: Penguin)
[5] Wong H, Radke C and Morris S 1995 *J. Fluid Mech.* **292** 71–94
[6] Yole-Dévelopement 2019 Microfluidic technologies are taking point-of-care testing to the next level *Molecular Medicine Tri-Conf.*
[7] Ma Y D, Li K H, Chen Y H, Lee Y M, Chou S T, Lai Y Y, Huang P C, Ma H P and Lee G B 2019 *Lab Chip* **19** 3804–14
[8] Volpatti L R and Yetisen A K 2014 *Trends Biotechnol.* **32** 347–50
[9] Williams J 2018 Technological developments in microfluidics—Andrew Demello (available at: www.global-engage.com/life-science/technological-developments-in-microfluidics-andrew-demello/)

[10] Williams J 2017 George whitesides on what's next for microfluidics—free presentation (available at: www.global-engage.com/life-science/george-whitesides-on-whats-next-for-microfluidics-free-presentation/)
[11] Patinglag L, Sawtell D, Iles A, Melling L M and Shaw K J 2019 *Plasma Chem. Plasma Process.* **39** 561–75
[12] Cao H, Zhou X and Zeng Y 2019 *Sens. Actuators* B **279** 447–57
[13] Luo G, Du L, Wang Y and Wang K 2019 *Particuology* **45** 1–19
[14] Shao M, Yu Q, Jing N, Cheng Y, Wang D, Wang Y D and Xu J H 2019 *Lab Chip* **19** 3974–8
[15] Demello A J 2006 *Nature* **442** 394–402
[16] Mao X and Huang T J 2012 *Lab Chip* **12** 4006–9
[17] Persat A *et al* 2015 *Cell* **161** 988–97
[18] Prakadan S M, Shalek A K and Weitz D A 2017 *Nat. Rev. Genet.* **18** 345
[19] Huber D, Oskooei A, Solvas X C I, Demello A and Kaigala G V 2018 *Chem. Rev.* **118** 2042–79
[20] Lee A P, Aghaamoo M, Adams T N and Flanagan L A 2018 *Curr. Stem Cell Rep.* **4** 116–26
[21] Lin L, Chen Q and Sun J 2018 *TRAC Trends Anal. Chem.* **99** 66–74
[22] Sanfilippo J E, Lorestani A, Koch M D, Bratton B P, Siryaporn A, Stone H A and Gitai Z 2019 *Nat. Microbiol.* **4** 1274–81
[23] Kinio S and Mills J K 2017 *IEEE Trans. Nanobiosci.* **16** 802–9
[24] Poceviciute R and Ismagilov R F 2019 *Nat. Biomed. Eng.* **3** 500
[25] Vedula E M, Alonso J L, Arnaout M A and Charest J L 2017 *PLoS One* **12** e0184330
[26] Sidar B, Jenkins B R, Huang S, Spence J R, Walk S T and Wilking J N 2019 *Lab Chip* **19** 3552–62
[27] Drieschner C, Könemann S, Renaud P and Schirmer K 2019 *Lab Chip* **19** 3268–76
[28] Hsu Y H, Liu W W, Wu T H, Lee C J T, Chen Y H and Li P C 2019 *Biomed. Microdevices* **21** 7
[29] Kaletta T and Hengartner M O 2006 *Nat. Rev. Drug Discovery* **5** 387–99
[30] Crane M M, Sands B, Battaglia C, Johnson B, Yun S, Kaeberlein M, Brent R and Mendenhall A 2019 *Sci. Rep.* **9** 9192
[31] Youssef K, Tandon A and Rezai P 2019 *Integr. Biol.* **11** 186–207
[32] Tian F, Feng Q, Chen Q, Liu C, Li T and Sun J 2019 *Microfluid. Nanofluidics* **23** 68
[33] Kinio S and Mills J K 2017 *Electrophoresis* **38** 1755–63
[34] Chen Y S, Ma Y D, Chen C, Shiesh S C and Lee G B 2019 *Lab Chip* **19** 3305–15
[35] Dixon C, Lamanna J and Wheeler A R 2020 *Lab Chip* **20** 1845–55
[36] Meier M, Kennedy-Darling J, Choi S H, Norstrom E M, Sisodia S S and Ismagilov R F 2009 *Angew. Chem., Int. Ed. Engl.* **48** 1487–9
[37] Neužil P, Giselbrecht S, Länge K, Huang T J and Manz A 2012 *Nat. Rev. Drug Discovery* **11** 620–32
[38] Courtney M, Chen X, Chan S, Mohamed T, Rao P P and Ren C L 2016 *Anal. Chem.* **89** 910–5
[39] Chen X and Ren C L 2017 *RSC Adv.* **7** 16738–50
[40] Zheng Y B, Kiraly B and Huang T J 2010 *Nanomedicine* **5** 1309–12
[41] Li L and Ismagilov R F 2010 *Annu. Rev. Biophys.* **39** 139–58
[42] Ferreira J, Castro F, Rocha F and Kuhn S 2018 *Chem. Eng. Sci.* **191** 232–44
[43] Liu D, Zhang H, Fontana F, Hirvonen J T and Santos H A 2018 *Adv. Drug. Deliv. Rev.* **128** 54–83
[44] Li Y, Xuan J, Hu R, Zhang P, Lou X and Yang Y 2019 *Talanta* **204** 569–75
[45] Hung L H and Lee A P 2007 *J. Med. Biol. Eng.* **27** 1
[46] Marre S, Park J, Rempel J, Guan J, Bawendi M G and Jensen K F 2008 *Adv. Mater.* **20** 4830–4
[47] Duraiswamy S and Khan S A 2009 *Small* **5** 2828–34
[48] Luo G, Du L, Wang Y and Wang K 2019 *Chem. Eng. Technol.* **42** 1996–2008
[49] Garg N, Tona R, Martin P, Martin-Soladana P, Ward G, Douillet N and Lai D 2020 *Lab Chip* **20** 1815–26
[50] Nette J, Howes P D and deMello A J 2020 *Adv. Mater. Technol.* **5** 2000060
[51] Ye Z, Wang K, Lou M, Jia X, Xu F and Ye G 2020 *Microfluid. Nanofluidics* **24** 38
[52] Rasouli M R and Tabrizian M 2019 *Lab Chip* **19** 3316–25
[53] Duncanson W J, Lin T, Abate A R, Seiffert S, Shah R K and Weitz D A 2012 *Lab Chip* **12** 2135–45
[54] Vladisavljević G T 2016 *Particuology* **24** 1–17
[55] Li W *et al* 2018 *Chem. Soc. Rev.* **47** 5646–83
[56] Karthikeyan K and Sujatha L 2018 *IEEE Sens. J.* **18** 5225–31
[57] Giménez-Gómez P, Baldi A, Ayora C and Fernández-Sánchez C 2019 *ACS Sens.* **4** 3156–65
[58] Subramanian V, Lee S, Jena S, Jana S K, Ray D, Kim S J and Thalappil P 2020 *Sens. Actuators* B **304** 127340
[59] Guan Y and Sun B 2020 *Microsyst. Nanoeng.* **6** 1–12
[60] Liu X, Wang Y and Song Y 2018 *Biosens. Bioelectron.* **117** 644–50
[61] Ortiz R, Chen J L, Stuckey D C and Steele T W 2019 *Micro Nano Eng.* **2** 92–103
[62] Yang K, Peretz-Soroka H, Liu Y and Lin F 2016 *Lab Chip* **16** 943–58
[63] Jain R, Thakur A, Kaur P, Kim K H and Devi P 2019 *TRAC Trends Anal. Chem.* **123** 115758
[64] Li Y, Chen Y, Yu H, Tian L and Wang Z 2018 *TRAC Trends Anal. Chem.* **98** 190–200
[65] Waheed A, Mansha M and Ullah N 2018 *TRAC Trends Anal. Chem.* **105** 37–51
[66] Ejeian F, Etedali P, Mansouri-Tehrani H A, Soozanipour A, Low Z X, Asadnia M, Taheri-Kafrani A and Razmjou A 2018 *Biosens. Bioelectron.* **118** 66–79
[67] Dalmieda J and Kruse P 2019 *Sensors* **19** 5134
[68] Yoshida J I, Nagaki A and Yamada T 2008 *Chem. Eur. J.* **14** 7450–9
[69] Duncombe T A, Tentori A M and Herr A E 2015 *Nat. Rev. Mol. Cell Biol.* **16** 554
[70] Yamaguchi H, Miyazaki M, Briones-Nagata M P and Maeda H 2010 *J. Biochem.* **147** 895–903
[71] Ahn G N, Yu T, Lee H J, Gyak K W, Kang J H, You D and Kim D P 2019 *Lab Chip* **19** 3535–42
[72] Chen W, Guo T, Kapoor Y, Russell C, Juyal P, Yen A and Hartman R L 2019 *Lab Chip* **19** 3628–40
[73] Martino C and deMello A J 2016 *Interface Focus* **6** 20160011
[74] Hassan S U, Gielen F, Niu X and Edel J B 2012 *RSC Adv.* **2** 6408–10
[75] Zhong J, Riordon J, Wu T, Edwards H, Wheeler A R, Pardee K, Guzik A A and Sinton D 2020 *Lab Chip* **20** 709–16
[76] Anna S L 2016 *Annu. Rev. Fluid Mech.* **48** 285–309
[77] Schmieder F, Schmieder S, Eger R, Friedrich S, Werner A, Danz N, Marx U and Sonntag F 2012 *Biomed. Eng./Biomed. Tech.* **57** 340–3
[78] Sun Q, Pei J, Li Q, Niu K and Wang X 2019 *Micromachines* **10** 849
[79] Yuen P K 2008 *Lab Chip* **8** 1374–8
[80] Perozziello G *et al* 2010 *Micro Nanosyst.* **2** 227–38
[81] Skafte-Pedersen P, Sip C G, Folch A and Dufva M 2013 *J. Micromech. Microeng.* **23** 055011
[82] Chronopoulou L, Sparago C and Palocci C 2014 *J. Nanopart. Res.* **16** 2703
[83] Pfreundt A, Andersen K B, Dimaki M and Svendsen W E 2015 *J. Micromech. Microeng.* **25** 115010

[84] Maillard D, De Pastina A, Larsen T and Villanueva L G 2019 *Rev. Sci. Instrum.* **90** 045006
[85] Chiadò A, Palmara G, Chiappone A, Tanzanu C, Pirri C F, Roppolo I and Frascella F 2020 *Lab Chip* **20** 665–74
[86] Longwell S A and Fordyce P M 2020 *Lab Chip* **20** 93–106
[87] Zhang H, Li G, Liao L, Mao H, Jin Q and Zhao J 2013 *Biomicrofluidics* **7** 034105
[88] Zhu J Y *et al* 2019 *Anal. Chem.* **91** 15784–90
[89] Uvet H, Hasegawa A, Ohara K, Takubo T, Mae Y and Arai T 2009 *IEEE Trans. Nanobiosci.* **8** 332–40
[90] Li G, Luo Y, Chen Q, Liao L and Zhao J 2012 *Biomicrofluidics* **6** 014118
[91] Yang H, Chen Z, Cao X, Li Z, Stavrakis S, Choo J, deMello A J, Howes P D and He N 2018 *Anal. Bioanal. Chem.* **410** 7019–30
[92] McMullen J P and Jensen K F 2010 *Annu. Rev. Anal. Chem.* **3** 19–42
[93] Garcia-Egido E, Spikmans V, Wong S Y and Warrington B H 2003 *Lab Chip* **3** 73–76
[94] Griffiths-Jones C M, Hopkin M D, Jönsson D, Ley S V, Tapolczay D J, Vickerstaffe E and Ladlow M 2007 *J. Comb. Chem.* **9** 422–30
[95] Goodell J R, McMullen J P, Zaborenko N, Maloney J R, Ho C X, Jensen K F, Porco J A Jr and Beeler A B 2009 *J. Org. Chem.* **74** 6169–80
[96] Koch K, van Weerdenburg B J, Verkade J M, Nieuwland P J, Rutjes F P and van Hest J C 2009 *Org. Process Res. Dev.* **13** 1003–6
[97] Sugimoto A, Fukuyama T, Rahman M T and Ryu I 2009 *Tetrahedron Lett.* **50** 6364–7
[98] Su Z, He J, Zhou P, Huang L and Zhou J 2020 *Lab Chip* **20** 1907–16
[99] Krishnadasan S, Brown R, Demello A and Demello J 2007 *Lab Chip* **7** 1434–41
[100] McMullen J P, Stone M T, Buchwald S L and Jensen K F 2010 *Angew. Chem., Int. Ed. Engl.* **49** 7076–80
[101] Srinivasan B and Tung S 2015 *J. Lab. Autom.* **20** 365–89
[102] Liao Z, Zhang Y, Li Y, Miao Y, Gao S, Lin F, Deng Y and Geng L 2019 *Biosens. Bioelectron.* **126** 697–706
[103] Chong Z Z, Tan S H, Gañán-Calvo A M, Tor S B, Loh N H and Nguyen N T 2016 *Lab Chip* **16** 35–58
[104] Teo A J, Yan M, Dong J, Xi H D, Fu Y, Tan S H and Nguyen N T 2020 *Microfluid. Nanofluidics* **24** 1–9
[105] Tan S H, Nguyen N T, Yobas L and Kang T G 2010 *J. Micromech. Microeng.* **20** 045004
[106] Gómez-Pastora J *et al* 2019 *J. Phys. Chem.* C **123** 10065–80
[107] Wu Y, Fu T, Ma Y and Li H Z 2015 *Microfluid. Nanofluidics* **18** 19–27
[108] Zeng W, Li S and Wang Z 2015 *Sens. Actuators* A **233** 542–7
[109] Crawford D, Smith C and Whyte G 2017 *Sci. Rep.* **7** 1–9
[110] Zeng S *et al* 2009 *Lab Chip* **9** 1340–3
[111] Gu H, Murade C U, Duits M H and Mugele F 2011 *Biomicrofluidics* **5** 011101
[112] Hou L *et al* 2014 *Anal. Methods* **6** 878–85
[113] Tangen U, Sharma A, Wagler P and McCaskill J S 2015 *Biomicrofluidics* **9** 014119
[114] Saez J, Etxebarria J, Antoñana-Diez M and Benito-Lopez F 2016 *Sens. Actuators* B **234** 1–7
[115] Girabawe C and Fraden S 2017 *Sens. Actuators* B **238** 532–9
[116] Feng S, Yi L, Zhao-Miao L, Ren-Tuo C and Gui-Ren W 2015 *Chin. J. Anal. Chem.* **43** 1942–54
[117] Lee C Y, Chang C L, Wang Y N and Fu L M 2011 *Int. J. Mol. Sci.* **12** 3263–87
[118] Ahmed H, Ramesan S, Lee L, Rezk A R and Yeo L Y 2020 *Small* **16** 1903605
[119] Yesiloz G, Boybay M S and Ren C L 2017 *Anal. Chem.* **89** 1978–84
[120] Lam R H and Li W J 2012 *Micromachines* **3** 279–94
[121] Woodruff K and Maerkl S J 2018 *Anal. Chem.* **90** 696–701
[122] Chong Z Z *et al* 2014 *Micro Nanosyst.* **6** 232–6
[123] Komoda T and Matsunaga T 2015 *Biochemistry for Medical Professionals* (New York: Academic)
[124] Nan L, Yang Z, Lyu H, Lau K Y Y and Shum H C 2019 *Adv. Biosyst.* **3** 1900076
[125] Dong X, Song P and Liu X 2019 *IEEE Trans. Nanobiosci.* **18** 373–80
[126] Nitta N *et al* 2018 *Cell* **175** 266–76
[127] Anagnostidis V, Sherlock B, Metz J, Mair P, Hollfelder F and Gielen F 2020 *Lab Chip* **20** 889–900
[128] Isozaki A *et al* 2020 *Lab Chip* **20** 2263–73
[129] Godino N, Pfisterer F, Gerling T, Guernth-Marschner C, Duschl C and Kirschbaum M 2019 *Lab Chip* **19** 4016–20
[130] Isozaki A *et al* 2020 *Sci. Adv.* **6** eaba6712
[131] Hayat Z and El Abed A I 2018 *Micromachines* **9** 183
[132] Paiè P, Zandrini T, Vázquez R M, Osellame R and Bragheri F 2018 *Micromachines* **9** 200
[133] Park S Y, Wu T H, Chen Y, Teitell M A and Chiou P Y 2011 *Lab Chip* **11** 1010–2
[134] Friese M, Nieminen T, Heckenberg N and Rubinsztein-Dunlop H 1998 *Nature* **394** 348–50
[135] Baigl D 2012 *Lab Chip* **12** 3637–53
[136] Zhang K, Liang Q, Ma S, Mu X, Hu P, Wang Y and Luo G 2009 *Lab Chip* **9** 2992–9
[137] Surenjav E, Priest C, Herminghaus S and Seemann R 2009 *Lab Chip* **9** 325–30
[138] Zhang K, Liang Q, Ai X, Hu P, Wang Y and Luo G 2011 *Lab Chip* **11** 1271–5
[139] Vashist S K, Luppa P B, Yeo L Y, Ozcan A and Luong J H 2015 *Trends Biotechnol.* **33** 692–705
[140] Noviana E, McCord C P, Clark K M, Jang I and Henry C S 2019 *Lab Chip* **20** 9–34
[141] Fu H, Song P, Wu Q, Zhao C, Pan P, Li X, Li-Jessen N Y and Liu X 2019 *Microsyst. Nanoeng.* **5** 1–12
[142] Phillips E A *et al* 2019 *Lab Chip* **19** 3375–86
[143] Yap B K *et al* 2018 *Sensors* **18** 2625
[144] Jenkins G and Mansfield C D 2013 *Microfluidic Diagnostics: Methods and Protocols* (Berlin: Springer)
[145] Kim C J, Ki D Y, Park J, Sunkara V, Kim T H, Min Y and Cho Y K 2020 *Lab Chip* **20** 949–57
[146] Lee K, Yoon T, Yang H S, Cha S, Cheon Y P, Kashefi-Kheyrabadi L and Jung H I 2020 *Lab Chip* **20** 320–31
[147] Nasseri B, Soleimani N, Rabiee N, Kalbasi A, Karimi M and Hamblin M R 2018 *Biosens. Bioelectron.* **117** 112–28
[148] Hemmig E, Temiz Y, Gökçe O, Lovchik R D and Delamarche E 2019 *Anal. Chem.* **92** 940–6
[149] Choi K, Ng A H, Fobel R and Wheeler A R 2012 *Annu. Rev. Anal. Chem.* **5** 413–40
[150] Nelson W C and Kim C J C 2012 *J. Adhes. Sci. Technol.* **26** 1747–71
[151] Barman J, Shao W, Tang B, Yuan D, Groenewold J and Zhou G 2019 *Micromachines* **10** 329
[152] Min X and Kim W S 2019 *Microfluid. Nanofluidics* **23** 127
[153] Leipert J and Tholey A 2019 *Lab Chip* **19** 3490–8
[154] Li J and Kim C 2020 *Lab Chip* **20** 1705–12
[155] Ding X *et al* 2013 *Lab Chip* **13** 3626–49
[156] Riaud A, Baudoin M, Thomas J L and Matar O B 2016 *IEEE Trans. Ultrason. Ferroelectr. Freq. Control* **63** 1601–7
[157] Renaudin A, Tabourier P, Zhang V, Camart J and Druon C 2006 *Sens. Actuators* B **113** 389–97
[158] Renaudin A, Tabourier P, Camart J C and Druon C 2006 *J. Appl. Phys.* **100** 116101
[159] Renaudin A, Sozanski J P, Verbeke B, Zhang V, Tabourier P and Druon C 2009 *Sens. Actuators* B **138** 374–82
[160] Wixforth A 2003 *Superlattices Microstruct.* **33** 389–96
[161] Agostini M, Greco G and Cecchini M 2019 *IEEE Access* **7** 70901–9

[162] Shilton R J, Mattoli V, Travagliati M, Agostini M, Desii A, Beltram F and Cecchini M 2015 *Adv. Funct. Mater.* **25** 5895–901
[163] Guttenberg Z, Müller H, Habermüller H, Geisbauer A, Pipper J, Felbel J, Kielpinski M, Scriba J and Wixforth A 2005 *Lab Chip* **5** 308–17
[164] Li Y *et al* 2012 *IEEE Trans. Semicond. Manuf.* **25** 323–30
[165] Zhang Y and Nguyen N T 2017 *Lab Chip* **17** 994–1008
[166] Long Z, Shetty A M, Solomon M J and Larson R G 2009 *Lab Chip* **9** 1567–75
[167] Hang Koh W, Seng Lok K and Nguyen N T 2013 *J. Fluids Eng.* **135** 021302
[168] Zhang Y and Wang T H 2013 *Adv. Mater.* **25** 2903–8
[169] Shikida M, Takayanagi K, Honda H, Ito H and Sato K 2006 *J. Micromech. Microeng.* **16** 1875
[170] Shikida M, Takayanagi K, Inouchi K, Honda H and Sato K 2006 *Sens. Actuators* B **113** 563–9
[171] Kim J A, Kim M, Kang S M, Lim K T, Kim T S and Kang J Y 2015 *Biosens. Bioelectron.* **67** 724–32
[172] Kanitthamniyom P and Zhang Y 2019 *Electrophoresis* **40** 1178–85
[173] Lu P H, Ma Y D, Fu C Y and Lee G B 2020 *Lab Chip* **20** 789–97
[174] Decrop D, Ruiz E P, Kumar P T, Tripodi L, Kokalj T and Lammertyn J 2017 Digital microfluidics assisted sealing of individual magnetic particles in femtoliter-sized reaction wells for single-molecule detection *Microchip Diagnostics* (Berlin: Springer) pp 85–101
[175] Liu Y and Papautsky I 2019 *Micromachines* **10** 107
[176] Dimov N, McDonnell M B, Munro I, McCluskey D, Johnston I, Tan C and Coudron L 2020 *J. Vis. Exp.* **156** e60489
[177] Wang Y, Ruan Q, Lei Z C, Lin S C, Zhu Z, Zhou L and Yang C 2018 *Anal. Chem.* **90** 5224–31
[178] Choi K *et al* 2013 *Anal. Chem.* **85** 9638–46
[179] Chiou P Y, Moon H, Toshiyoshi H, Kim C J and Wu M C 2003 *Sens. Actuators* A **104** 222–8
[180] Pei S N, Valley J K, Wang Y L and Wu M C 2015 *J. Lightwave Technol.* **33** 3486–93
[181] Collier C M, Hill K, DeWachter M A, Huizing A M and Holzman J F 2015 *J. Biomed. Opt.* **20** 025004
[182] Thio S K, Bae S and Park S Y 2020 *Sens. Actuators* B **308** 127704
[183] Park S Y, Teitell M A and Chiou E P 2010 *Lab Chip* **10** 1655–61
[184] Jiang D, Lee S, Bae S W and Park S Y 2018 *Lab Chip* **18** 532–9
[185] Lee S, Thio S K, Park S Y and Bae S 2019 *Harmful Algae* **88** 101638
[186] Decrop D *et al* 2016 *Anal. Chem.* **88** 8596–603
[187] Unger M A, Chou H P, Thorsen T, Scherer A and Quake S R 2000 *Science* **288** 113–6
[188] Thorsen T, Maerkl S J and Quake S R 2002 *Science* **298** 580–4
[189] Lee C C *et al* 2005 *Science* **310** 1793–6
[190] Araci I E, Robles M and Quake S R 2016 *Lab Chip* **16** 1573–8
[191] Laval C, Bouchaudy A and Salmon J B 2016 *Lab Chip* **16** 1234–42
[192] Levi T and Fujii T 2016 *Micromachines* **7** 146
[193] Huang W Y, Liu C A, Fan R S, Lin Z D, Wang K and Lee G B 2017 *Biomicrofluidics* **11** 034109
[194] Padmanabhan S, Misteli T and DeVoe D 2017 *Lab Chip* **17** 3717–24
[195] Yap Y C, Dickson T C, King A E, Breadmore M C and Guijt R M 2017 Microfluidic device for studying traumatic brain injury *Stem Cell Technologies in Neuroscience* (Berlin: Springer) ch 10, pp 145–56
[196] Yu F B, Willis L, Chau R M W, Zambon A, Horowitz M, Bhaya D, Huang K C and Quake S R 2017 *BMC Biol.* **15** 11
[197] Liu W T, Lee W B, Tsai Y C, Chuang Y J, Hsu K F and Lee G B 2019 *Biomicrofluidics* **13** 014114
[198] Huang S P, Chuang Y J, Lee W B, Tsai Y C, Lin C N, Hsu K F and Lee G B 2020 *Lab Chip* **20** 1103–9
[199] Lai A, Altemose N, White J A and Streets A M 2019 *J. Micromech. Microeng.* **29** 107001
[200] Lee Y S, Bhattacharjee N and Folch A 2018 *Lab Chip* **18** 1207–14
[201] Brower K, Puccinelli R R, Markin C J, Shimko T C, Longwell S A, Cruz B, Gomez-Sjoberg R and Fordyce P M 2018 *HardwareX* **3** 117–34
[202] Tanaka Y, Fujikawa T, Kazoe Y and Kitamori T 2013 *Sens. Actuators* B **184** 163–9
[203] Balck A, Michalzik M, Al-Halabi L, Dübel S and Büttgenbach S 2011 *Sens. Transducers* **127** 102
[204] Wang Y *et al* 2019 *Microfluid. Nanofluidics* **23** 112
[205] Clime L, Daoud J, Brassard D, Malic L, Geissler M and Veres T 2019 *Microfluid. Nanofluidics* **23** 29
[206] Pekas N, Zhang Q and Juncker D 2012 *J. Micromech. Microeng.* **22** 097001
[207] Khoshmanesh K, Tang S Y, Zhu J Y, Schaefer S, Mitchell A, Kalantar-Zadeh K and Dickey M D 2017 *Lab Chip* **17** 974–93
[208] Cybulski O, Jakiela S and Garstecki P 2016 *Lab Chip* **16** 2198–210
[209] Churski K, Kaminski T S, Jakiela S, Kamysz W, Baranska-Rybak W, Weibel D B and Garstecki P 2012 *Lab Chip* **12** 1629–37
[210] Postek W, Kaminski T and Garstecki P 2017 *Analyst* **142** 2901–11
[211] Wong D and Ren C L 2016 *Lab Chip* **16** 3317–29
[212] Wong Y H 2016 Feedback controls in droplet microfluidics *Master's Thesis* University of Waterloo
[213] Hébert M, Courtney M and Ren C L 2019 *Lab Chip* **19** 1490–501
[214] Wong D, Erkorkmaz K and Ren C 2020 *IEEE/ASME Trans. Mechatronics* **25** 1129–37
[215] Wang C, Nguyen N T, Wong T N, Wu Z, Yang C and Ooi K T 2007 *Sens. Actuators* A **133** 323–8
[216] Thurgood P, Zhu J Y, Nguyen N, Nahavandi S, Jex A R, Pirogova E, Baratchi S and Khoshmanesh K 2018 *Lab Chip* **18** 2730–40
[217] Thurgood P, Suarez S, Chen S, Gilliam C, Pirogova E, Jex A R, Baratchi S and Khoshmanesh K 2019 *Lab Chip* **19** 2885–96
[218] Gao R Z, Hébert M, Huissoon J and Ren C L 2020 *HardwareX* **7** e00096