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Recent developments of microfluidics as a tool for biotechnology and microbiology

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Academic microfluidics has decisively shifted in recent years from the research on phenomenology and proof-of-concept fluidic functionalities to the developments oriented at applications with biology, medicine and biotechnology in prime focus. Significant efforts are made to demonstrate that microfluidics can be used in unspecialized laboratories to perform previously mundane tasks faster and easier, or to venture into new research areas that were unavailable or unattractive when only classical means of microbiology or biotechnology were employed. Here we review a variety of biological experiments recently performed in microfluidic assays. We categorize the microfluidic systems by the key role they play in the biological experiments as: (i) controlled reaction chambers, (ii) high-throughput arrays, or (iii) micro-positioning systems. We also discuss the outlook for further development and applications of microfluidics in biological sciences.

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Intro

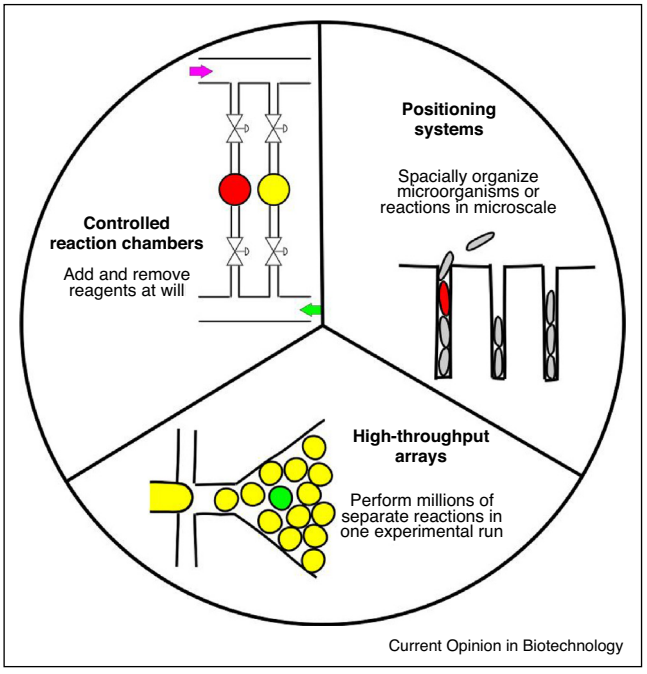
Microfluidics has been in development for decades, however the pacing at which new microfluidic platforms are presented has increased in the recent years. Engineers and scientists have been producing ever more advanced instruments for myriads of operations on liquids at the microscale [1,2]. Microfluidic tools enable researchers to manipulate minute volumes of liquids in a precise, automated and controlled manner that allows for performance of multiple experiments in parallel and minimizes the errors introduced by manual labor. Miniaturization enables reduction of the experiment volume, thus diminishing the potential reagent cost and reaction time. Microfluidic systems can be fabricated with resolution of single microns or better. This excellent precision of production enables designs that can hold single cells in one place or that can guide the cells to a desired location, while the whole experiment can be constantly monitored [1,3,4].

It has become clear that microfluidics could provide tools that would open new exciting directions of research in other fields. This is especially true for biology, where microfluidics allows for example, manipulations of single cells or monitoring of the enzymatic reaction rates at an unprecedented scale — both in the sense of a small physical size but also in the immense number of experiment repetitions and assay execution rate. In this review, we discuss the most recent advancements in biological sciences that use microfluidic platforms to achieve goals that would otherwise be very difficult or impossible to fulfill. We organize them into three categories according to the key role played by microfluidics: (i) controlled reaction chambers, where microfluidics offers precise control over reaction conditions at the micro-scale, (ii) high-throughput arrays, where microfluidics facilitates parallelization and offers access to pool sizes that would otherwise be virtually impossible to achieve, and (iii) positioning systems, where microfluidics is the key enabling technology to localize cells, groups of cells or tissues at designated areas (Figure 1).

Controlled reaction chambers

The basic function of microfluidic systems here is to guide and switch the flow of reagents in small channels and chambers. This basic functionality is important as it allows for a precise control over the chemical environment for the cells and organisms either treated or cultured in small chambers. One influential component in this respect is the integrated pneumatic microvalve (often called 'Quake valve' after Stephen Quake — the lead author of the technology). Using Quake-valves Kim *et al.* designed a microfluidic chip with 96 parallel channel circuits for automated sample preparation for microbial genomics (Figure 2a). The key steps in the NGS sample preparation protocol were automated and optimized, so that the DNA input could be reduced 100-fold. Movements of reagents were provided by subsequent operations of the valves [5]. Similar fluid handling system was used by Glick *et al.* in their microfluidic human membrane protein (HMP) array. In their device different membrane proteins are assembled *in situ* in designated microfluidic chambers via on chip *in vitro* protein expression (Figure 2b). Notably, this technology allowed for simultaneous screening of ~2100 different HMP-s on chip and

Figure 1



Three categories of microfluidic approaches based on their main experimental role: (i) with controlled reaction chambers researcher can handle multiple experimental steps on single device, (ii) in high-throughput arrays parallel experiments can be conducted in vast numbers and (iii) in positioning systems analyzed species or molecules are positioned accurately for manipulation and analysis.

investigating their interactions with a portfolio of viruses [6•]. Microvalves can also be used in more heterogeneous bioassays: complexes of transcription factors and DNA were pushed towards surfaces covered with antibodies. Later sequencing of the DNA enabled characterization of binding motifs of transcription factors [7].

The mixing of reagents can also be actuated mechanically by rearrangement of whole channels and chambers without any complex automation. In the SlipChip technology the sample is compartmentalized by slipping two plates imprinted with wells and ducts to convert continuous fluidic paths into isolated chambers. SlipChips can be operated by hand, which makes this technology attractive for laboratories not specialized in microfluidics. SlipChip has found use in digital nucleic acid amplification applications like digital PCR [8], HIV load quantification [9] and a very innovative use of digital LAMP assay to screen for the antibiotic susceptibility of bacteria in less than 30 min [10•].

Sets of effectively isolated experiments can be conducted in microfluidic systems also without valves or solid barriers between the reaction chambers. For example, cells can be simply fixed in hydrogel that warrants their separation from each other during serial exchange of reagents. This undemanding and efficient approach was applied to multiple displacement amplification (dMDA) of purified DNA templates, cultured bacterial cells and human microbiome samples. Subsequent sequencing of single-cell MDA products showed good coverage uniformity and a reduced chimerism in comparison with bulk preparation protocols [11].

Complex biological systems can be constructed by connecting multiple microfluidic units, and this is being done in the field of organs-on-chips, which might lead to, for example, toxicology assays *in vivo* without actual living organisms being drugged. For example, Zhang *et al.* built a modular automated multiorgan-on-a-chip platform. They used the device to culture hepatic and cardiac organoids. Such devices serve as miniaturized 3D human organ models that in their study was used for drug toxicity screening [12]. To get a more detailed insight into recent progress in using organs-on-chip and organoids-on-chip technology for modeling human physiology and disease conditions we recommend the following review [13].

Microfluidic devices enable carrying out advanced experiments also away from the laboratories. Lambert *et al.* demonstrated an array of wells that were connected to the environment by microfluidic channels. After deploying the assay in seawater, chemo-attractants lured microorganisms into the wells, allowing for *in situ* analysis of marine microbial ecosystems [14].

High-throughput arrays

Massive parallelization of experiments provides for excellent statistical data, screening of large numbers of compounds, or for seeking rare events in large pools of molecules or organisms. Increasing the throughput of arrays has been classically done in microwell arrays, which are still being developed and integrated with microfluidic flow chambers to achieve, for example, 150 000 wells of 100 pl volume [15]. There is an ongoing trend to improve on microwell systems by replacing the wells with static droplets printed on surfaces. Printed droplet platforms can be either highly sophisticated arrays with thousands of picoliter compartments printed on a plate that is integrated with electrodes [16], or they can be designed for simplicity directed towards democratization of the microfluidic technologies [17]. A combination of a microwell array with a droplet assay was used for analysis of effects of more than 80 000 combinations of drugs on bacteria, revealing multiple synergistic interactions that could point towards effective therapy of drug-resistant bacteria [18]. However, by far the most developed branch of high-throughput microfluidic arrays is droplet microfluidics [4].

In droplet assays, each droplet forms an aqueous environment isolated by the surrounding oil phase. If diffusional transport of particles between the droplets is under