Chapter 10
Trends and Perspectives

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10.1 Summary of Chapters

Throughout the book chapters, researchers have highlighted the recent advancement in microfluidic areas, particularly those involving microdroplets.

Simon and Lee focused on microfluidics droplet manipulations and applications, including droplet fusion, droplet fission, mixing in droplets, and droplet sorting. By combining these operations, they have shown promising applications in executing chemical reactions and biological assays at the microscale.

Day and Karimiani discussed dropletisation of bio-reactions.

Zhang and Liu elaborated the physics involved in multiphase flows and microdroplets dynamics. They emphasized the important dimensionless parameters relating to droplet dynamics with droplet generation process as an example.

Barber and Emerson discussed the fundamental droplet handling operations and the recent advances in electrowetting microdroplet technologies. They also provided an overview of droplet-based electrowetting technologies in biological and chemical applications.

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P. Day et al. (eds.), *Microdroplet Technology: Principles and Emerging Applications in Biology and Chemistry*, Integrated Analytical Systems,
DOI 10.1007/978-1-4614-3265-4_10,

Droplet-based microfluidics as a biomimetic principle in diagnostic and biomolecular information handling were highlighted by Köhler addressing potential of applying segmented fluid technique to answer to the challenges of information extraction from cellular and biomolecular systems.

Using the flow rates, applied pressures, and flow rate ratios in a closed feedback system, the active control of droplet size during formation process in microfluidics was achieved by Nguyen and Tan.

Velev, Petsev, and Chang discussed droplet microreactors for materials synthesis. They briefly described microfluidics for droplet generation as well as fabrication technology. They provided detail study of transport in microchannels and droplet microfluidics for mesoporous particle synthesis.

Kaminski, Churski, and Garstecki reviewed the recent advances in building modules for automation of handling of droplets in microfluidic channels, including the modules for generation of droplets on demand, aspiration of samples onto chips, splitting and merging of droplets, incubation of the content of the drops, and sorting.

Zagnoni and Cooper have demonstrated the use of on-chip biocompatible microdroplets both as a carrier to transport encapsulated particles and cells, and as microreactors to perform parallel single-cell analysis in tens of milliseconds.

10.2 General Situation

Here we try to explore the technology development cycle and market trend for microfluidics devices. Microfluidic systems were first pioneered by Stanford's research introducing a chromatography chip about 30 years ago [1]. It was probably too ahead of time, yet only 15 years later, an avalanche of microfluidics developments was triggered by Manz's group [2] introduction of on-chip capillary electrophoresis (CE). This technology went through a Gartner hype cycle as illustrated in Fig. 10.1. Manz's CE chip resulted in a technology trigger to lead to inflated expectations in the late nineties for microfluidics, mirroring the Silicon Valley Technology bubble hype. Since then, there have been thousands of researchers developing microfluidic systems for various applications and with different goals. [3] Some were interested in basic research, some in commercial applications. However, very few of them were commercially successful in finding the ground-breaking applications. Microfluidics failed to deliver the initial promises to provide a revolutionary technology platform for life sciences and hence disappointed investors. So far, the most successful droplet microfluidics device is the inkjet printer; the commercialization of other miniaturization technology remains highly attenuated even though some areas have made good progress, such as Caliper's LabChip. Why is it that with such tremendous effort there is so little outcome? Let's analyze the reasons for the slow adoption of this promising enabling platform technology. We will further discuss if this technology is close to finding the "holy-grail" of analytics despite the past disappointing track record.

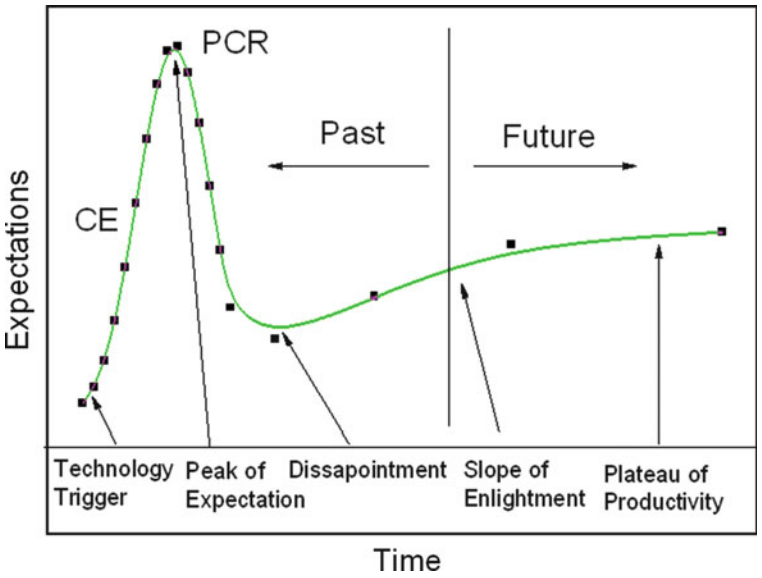

Fig. 10.1 Gartner Hype Cycle for Microfluidic Technology. The development of the capillary electrophoretic (CE) chip initially triggered the technology development. An example of a device produced during the peak expectation phase is exemplified by the micro polymerase chain reaction (PCR) system. After multiple disappointments currently the technology has now entered the slope of enlightenment

10.3 Scientific and Technology Origin

The chosen approach to demonstrate the value of microfludic applications contrary most likely is the major problem. Microfluidic systems have not been developed based on industrial or applications demand. These systems are mostly based on "leftover" manufacturing equipments and tools from the semiconductor industry. Using a push-pull analogy, microfluidics systems are "pushed" by manufacturers rather than "pulled" from market demand. The semiconductor industry follows the well known Moore's law, increasing wafer size, and shrinking device dimensions. The industry constantly needs to invest huge amounts of capital equipment with a short technology advancement cycle. In order not to obsolete the costly equipment, device manufacturers found microelectromechanical systems (MEMS) attractive. It is economical to convert the outdated integrated circuits (IC) production lines to produce MEMS devices such as pressure sensors, accelerometers etc. Meanwhile, integrated MEMS devices are also following Moore's law, although somewhat delayed in comparison to the ICs. Therefore, further converting such production lines to make microfluidic devices becomes the next natural option. The critical dimensions of these devices are well within the capability of existing semiconductor equipment and they are relatively simple to make. They need only a few fabrication

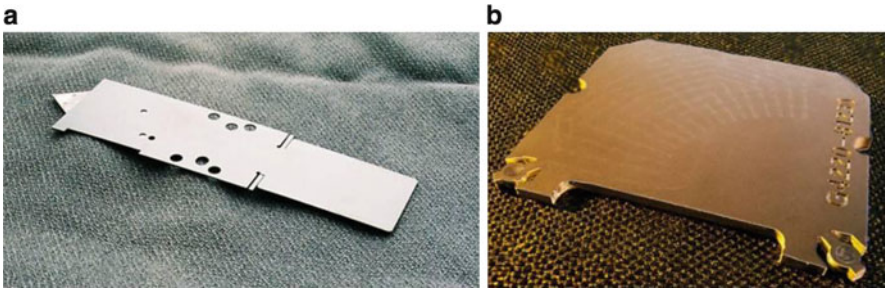

Fig. 10.2 Agilent chip device for (**a**) mixer from 6 layers of stainless steel and (**b**) LC-MS from Polyimide. Both devices are used in commercial products for proteomic mass spectrometry and for ultra high pressure liquid chromatography, respectively

steps, with contact printing for lithography often proving to perfectly suffice. The only special tool usually required is the wafer bonder, as well as the availability of etching method for glass. Next comes the basic question: who wants these devices and why? One of the fundamental problems of microfluidic devices not being commercially successful is rooted in the simple fact that they were NOT developed based on market demand, but quite contrarily. Such a starting point was risky as microfluidics development was often used to justify longer lifetime of leftover and aged IC facilities. Then the problem became how to find the applications and market demand for those devices. "Retrofitting" is well documented to rarely work.

Fortunately, there are now researchers who adopted the right approach. A new age of microfluidics devices for heat exchanging, mixing, and subsequent high performance liquid chromatographic (HPLC) separations are offered for example by Agilent based on the application demand for the device with specific performance in the market place, not to just redeplying old fabrication production line. These microfluidic devices are made of six layers of stainless steel cut by laser and glued together (see Fig. 10.2a). The devices are cheap, reliable, and able to withstand high pressure. To make them more user-friendly, the device extensions for connection can be bent to different angles based on application demand. Also previously a version of the HPLC chip that incorporated sample preparation was made from polyimide using printed circuit board (PCB) technology (see Fig. 10.2b). It enabled integration of heaters to locally control temperature.

Other fabrication techniques for microfluidics started to emerge, such as polymer-based microfluidics using polydimethylsiloxane (PDMS). The PDMS process is simple and it does not even require a well equipped cleanroom. Nevertheless the material itself is permeable to certain molecules which brings other problems making PDMS devices less competitive. Injection molding and hot embossing (imprinting) are other examples of different approaches compared to employing the silicon wafer processing facilities.

Are there any other problems with microfluidics? Firstly there is a scaling law which predicts problems for quantitative molecular detection limits at the nanometer scale. Every technique has a detection limit requiring a certain number of

molecules to be presented. This limit is not altered with the sample size, i.e., very small samples have to be highly concentrated to be exceed the limit of detection. This makes theses samples too concentrated to be of any interest. A restriction to pure compounds, or at the percentage level, seems to be interesting for more academic research only. Optimal fluidic dimensions for practical analytical chemistry look like to be from about 5–50 μm. That is a problem but still does not explain why the microfluidics devices are not flooding the market and why they have not "wiped out" conventional systems.

10.4 Example: PCR on Chip

Perhaps we can now analyze one popular microfluidics device as an example: miniaturized polymerase chain reaction system (microPCR). This process was first demonstrated by Northrup in 1993 and since then, hundreds of research groups have been designing their own systems in highly innovative approaches. However, none of them has been commercially introduced. The initial incentive seems very simple: the microPCR needs to be small so that it only requires the use of very small amount of reagents making the PCR economical. Surprisingly, in reality that is not always an advantage even though some researchers like to claim so. PCR is so sensitive that it can detect only a few molecules of DNA or RNA. Smaller amount of reagents indeed brings the cost down but the negative effect is that it decreases the risk of detection reliability through lowering the sample volume. Typically, a sample with volume from 5 μL to 10 μL can be used to detect one molecule of DNA. Using 10 μL for comparison, if the sample is split into 100 units with 100 nL each, then on the average the DNA concentration has to be increased 200 times to have a single DNA molecule in each sample. In reality that means that we are losing sensitivity by lowering sample volume making it unsuitable for direct diagnoses of infectious diseases. There are two exceptions, one is digital PCR [4] and the other one is PCR with sample pre-concentration [5]. Digital PCR divides one sample into into hundreds or thousands of tiny wells. It is based on exploiting use of sample dilution so extreme that a significant number of wells will intentionally receive no DNA while others gain a single DNA template to seed the PCR. The count of amplified wells determines the absolute number of DNA molecules in the original sample, making this PCR system intrinsically quantitative. That is an excellent approach and one that is specifically enabling through miniaturization. The only drawback is that for many applications quantitative PCR is not always required, and therefore the digital PCR is often overkill. However should quantitative PCR prove to be necessary, digital PCR could provide the answer.

A second case where the sample can be small is shown in Pipper's work as they run a pre-concentration step prior to PCR itself. His starting volume was only 40 μL compared to conventional Qiagen protocol requiring volume of 140 μL. Nevertheless he was able to run real-time RT-PCR with only a 100 nL sample volume while achieving two cycles smaller critical threshold, demonstrating that a small volume of PCR sample can be used for diagnostics without sacrificing the limit of detection.

This brings us to another problem which is working with clinical samples. These assays typically require binding of active component such as protein or DNA/RNA to achieve immobilization, washing off most of unwanted substances, and eventually release of the active component for further processing. A typical 140 μL volume of clinical sample as mentioned before is far too large to fit inside a micromachined microfluidic system. Also some reagents have to be stored separately from each other as well as outside the microfabricated device. If the sample as well as the reagents have to be stored separately (most likely in plastic devices), is there any justification of using the microfabricated device itself? Some researchers believe that the whole system can be produced by injection molding, such as GenExpert from Cepheid [6] which is one of the very few commercially available systems performing fully automated sample preparation followed by real-time PCR. A different approach was taken by Veredus Laboratories. They followed a previously described path of using outdated semiconductor process from ST Microelectronics to make advanced PCR systems with in situ hybridization [7]. The system is more labor intensive than sample-to-answer system such as GenExpert, but it is capable of identifying numerous genes simultaneously, offering advantage when screening for a few closely related pathogenic strains or detection of pathogens for homeland security applications.

10.5 Economical

From a technology development cycle perspective, there are other reasons why adoption of microfluidics technology is so slow.

Reason number one is the lack of economy-of-scale. In order for any technology to take off, it has to reach the tipping point in the market place to inflame the "viral effect" that triggers a high volume need; in economic terms, the economy-of-scale has to be in place. Without high volume it is hard to reduce manufacturing cost, and without an affordable price, it is hard for the new technology to be widely adopted. It is known as the "chasm" in the technology adoption cycle [8]. It becomes a "chicken-and-egg" dilemma. So what are the potential high volume markets? Over the years we have seen increasing rate of adoption of biological research helped by droplet microfluidic devices as tools. Examples of such significant progress are HPLC [9], "fluidic transistors" by Cytonix [10], and high throughput screening of biological reactions [11]. Digital microfluidics using "fluidic transistors" has potentially wide applications in diagnostic, chemical detection, bio-sequencing and synthesis as well as tissue engineering. The strong growing demand for fast, reliable, repeatable, and cost-effective biological analysis and diagnostic systems has driven the development of such systems. Microfluidic systems have been proven to be an enabling technology platform, benefitting through extensive research performed over years of exploration. However, currently, the devices were individually researched and prototyped by many academic research groups or small commercial groups. Each device has individual fabrication steps and choice of materials. It is lack of a "standard" manufacturing process which prevents

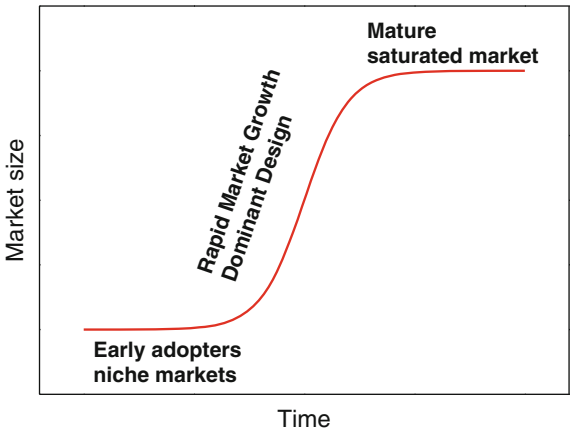

Fig. 10.3 Innovation life cycle

large scale repeatable production, therefore lack of the momentum of building critical mass towards the tipping point. High capital investment and low return on capital becomes the barrier. In our opinion, the commercialization community of microfluidic systems needs to converge on to adopting "standard" materials and manufacturing techniques. Interestingly with digital microfluidics we start to see the genesis of such a trend. Digital microfluidics has become a flexible platform for various bioprocessing and bioanalytical applications.

Reason number two is the co-development of supporting and companion technologies such as detection systems. Often, microfluidic devices are not stand alone as ready-to-use systems, they need to be integrated with other devices to form the complete system for given applications. If a technology platform is too ahead of its prime time, it will lack the associated supporting infrastructure, thus it would be suppressed until the companion technologies catch up. For example, in the case of microfluidic diagnostic devices such as micro real-time PCR, there is need for miniature reliable optical sensing devices and signal processing. In the past 10 years, CCD imaging and digital signal processing have made tremendous progress to make fast, reliable, and cost-effective diagnostic system possible.

Reason number three is the socio-economic environment. In the past 20 years, the bioscience community focused efforts on finding drugs for treating diseases. Now there is a political-social-economical shift towards early disease diagnoses and prevention to reduce the rapid increase in healthcare burden due to expensive treatment. Microfluidic systems have proven to be critical building blocks for bioanalysis and diagnostic instrumentation, and some of the devices have shown potential to be the consumer product for environmental monitoring and pandemic prevention diagnostic tools [5]. Also, for any technology platform, during the early development stage, there is need for enthusiasm from visionaries and investors. In the past 10 years, the venture capital community shifted investment strategy towards emerging markets, which reduced the early stage technology platform survival rate in

developed countries due to lack of funding to turn the corner on the s-curve of innovation life cycle (see Fig. 10.3). Nowadays, the situation starts to turn around.

Successful introduction of droplet microfluidics into the market requires scenario analysis in the early stage of the product development cycle, as would be expected for any other product development. The purpose is to identify the key drivers in the application market place and uncertainties, then to come up with several scenarios and corresponding technology trends so that the likelihood for commercial success can be more precisely recommended. Here the key drivers are cost effectiveness, high sensitivity, reliability, high-speed, and portability to perform bioanalysis. Cost effectiveness requires small sample volume and here the microfluidics has its place. The key uncertainties of the product development are convergence of repeatable large scale manufacturing techniques, macro-economic condition, and the emerging and development of competing technologies. As an example we can look at severe acute respiratory syndrome (SARS) [12] pandemic diagnostic market in 2003. At the time of the SARS pandemic the diagnoses was performed at specialized well equipped clinics and hospitals, e.g., in Singapore with its 4.5 million population all SARS testing was conducted only at Tan Tock Seng Hospital using laboratory-sized PCR systems. Luckily the early symptom of SARS is the onset of fever which could be detected by ultra fast infrared (IR) cameras. This mass testing practically eliminated the SARS virus spreading. This pandemic serves as a wakeup call. What would happen if technology such as offered by the IR camera is not effective for future pandemic? It is the perfect opportunity for microfludic technology to be implemented into a product that can penetrate consumer market. From this example we can see the importance of scenario analysis to spot the trend ahead of the market need and the necessary layout corresponding strategy for technology commercialization.

10.6 Outlook

Our previous discussion and overview may look pessimistic, but in fact we are just trying to identify the reasons why, in spite of a lot of efforts, the results are still evasive. So now comes the question: what kind of future awaits microfluidics? There are many examples of new technologies which looked so promising but soon were forgotten. Will microfluidics follow such a path? We believe that most likely this will not be happening. There are areas where microfluidics will eventually be the dominant if not the only technology. Obvious prime applications are anything with volume or weight limitations, for example, in space program applications [13] where weight limit is the dominate factor that filters out the conventional approaches. We can envision remotely controlled system for Moon or Mars exploration, that in microfluidics-based technology will be top candidate for any diagnostic and analytical tool due to its small volume and corresponding light weight. Besides these rather exotic systems, where else could microfluidics prevail? We have already mentioned digital PCR and surface-based virtual reaction chambers (VCRs). Their

advantages are obvious: digital PCR can be used to determine absolute number of DNA copies in the original sample and VCR-based systems cost only a few cents. What else? Of course, capillary electrophoreses is an example, liquid and gas chromatography and heat exchanger/mixers are also available and successfully marketed by Agilent. We can expect further development in these fields.

Further, we envision three major streams of future development apart from currently existing commercially successful devices.

A first stream could be massively parallel systems for drug or patient screening that are capable of competing with fully automated robotic systems used by big centralized hospital laboratories. Here the cost of the microfluidics is not critical because it is orders of magnitude cheaper than the current robotic approaches. An example of this approach is Steve Quake's massively parallel system [14]. Also Affymetrix's DNA chip [15] probably fits into this category.

A second stream could be simple microfluidic devices for point-of-care applications, where the cost of both capital investment and cost per test are of utmost importance. Here the microfluidics technology will compete with injection molding which naturally brings up a question, if there is even a chance that microfluidics can to win this contest. Injection molded parts are so cheap that their disposability is more economical than any attempt of cleaning the parts and reuse them. From a practical point of view, when it comes to clinical diagnostics, the doctors firmly insist on disposable devices to maintain an absolute sterile environment for the assay, and reduce ambiguity of determining results. This poses serious cost issue to microfluidics because currently they are just too expensive. Even channel free systems such as surface-based microfluidics relying on electrowetting is too costly. They are actually very interesting examples of versatile microfluidics systems due to the fact that they can be easily reprogrammed so that the layout of the microfluidics channel can be quickly changed. However, the reality is that for routine testing/diagnoses we do not need to change the microfluidics layout because there are simple techniques to achieve it so the versatility is not always needed. In this case, the technology can be considered overkill.

There are other competing techniques such as droplet-based PCR [16] which is based on single step lithography and simple heater. It can be probably further simplified to either use stamping (as shown schematically in Fig. 10.4) or eliminate requirement for lithography.

Another example is emulsion PCR [17]. Here the PCR is performed on beads each containing only single template molecule. Each bead is enclosed in a tiny sample droplet with PCR master mix and the thermal cycling is performed inside the droplet. The advantage of this system is that typically there is only a single DNA molecule and single bead within each droplet thus eliminating interference with other DNAs. Once the PCR is completed the emulsion is spread over a picowell plate reader. The size of each well is only 40 μm and beads 28 μm forming a system where there is only a single bead residing within each well which is enabling of single molecule sequencing.

A third stream of microfluidic devices is used for cell biology and tissue engineering research support [18]. Here the high cost of the microfluidic systems

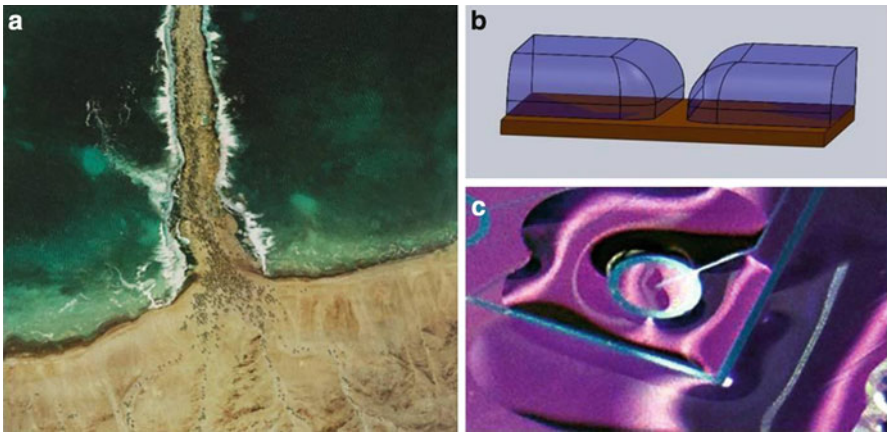

Fig. 10.4 (**a**) Satellite picture reconstruction of Moses leading his people across the Red Sea (copyright by The Glue Society, Australia, reprinted with permission), at this scale it is very unlikely, but nevertheless it is "an incredible story" showing that hydrostatic forces are dominated by surface tension. Inspired by earlier Takehiko Kitamori's presentation we show here schematically (**b**) device with two regions separated from each other by a hydrophilic/hydrophobic surface patterning. It can be relatively easily achieved at the micron scale where surface tension is much greater than hydrostatic forces. (**c**) Photograph of an actual device based on hydrophilic/hydrophobic concept

for research is tolerated as long as really novel effects or information can be achieved. So far cell biology is supported by microfluidics in areas of protein crystallization, stem cell sorting and differentiation, embryo handling [19] structured tissue engineering as well as regenerative medicine. One typical example is seeding stem cells on a scaffolding to form a bioartificial microreactor, such as kidney [20] or liver. Also potential patients would clearly benefit from bioartificial organs such as kidney which would eliminate their frequent visits of dialysis centers improving quality of their lives.

There will always be niche areas where microfluidics could play an important role, such as digital PCR for quantitative molecular testing for routine medical diagnostic.

Overall there is definitely light at the end of the tunnel but it will take some time to get there.

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