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Microfluidics and the life sciences

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ABSTRACT

The field of microfluidics, often also referred to as "Lab-on-a-Chip" has made significant progress in the last 15 years and is an essential tool in the development of new products and protocols in the life sciences. This article provides a broad overview on the developments on the academic as well as the commercial side. Fabrication technologies for polymer-based devices are presented and a strategy for the development of complex integrated devices is discussed, together with an example on the use of these devices in pathogen detection.

Keywords: *microfluidics, Lab-on-a-Chip*

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Introduction

As the microelectronic revolution changed the way in which electronic components and circuits were manufactured 50 years ago that led to an explosive growth in the applications of integrated circuits and a birth of new industries, a similar development can be seen with the introduction of miniaturisation in the life sciences with the initial concept of the so-called "miniaturised total analysis system" (μ-TAS), also often called "Lab-on-a-Chip" technology. This deals with the handling and manipulation of miniature amounts of liquid in analyses conducted within the Life Sciences research and was introduced about 20 years ago[1]. The first review to describe this technology appeared in *Science Progress* about 15 years ago[2]. We now discuss how the field has progressed and which of the early promises have so far have been fulfilled and which still belong to the realms of science-fiction.

Recent years have witnessed an explosive growth of scientific activities in the Lab-on-a-Chip technology. As illustrated in Figure 1, the number of scientific publications within the "microfluidics" area[3] has increased from 38 to 1270 between 2000 and 2010. However, the progress in commercialising microfluidics-enabled products has been much slower than anticipated in the early years[2,4].

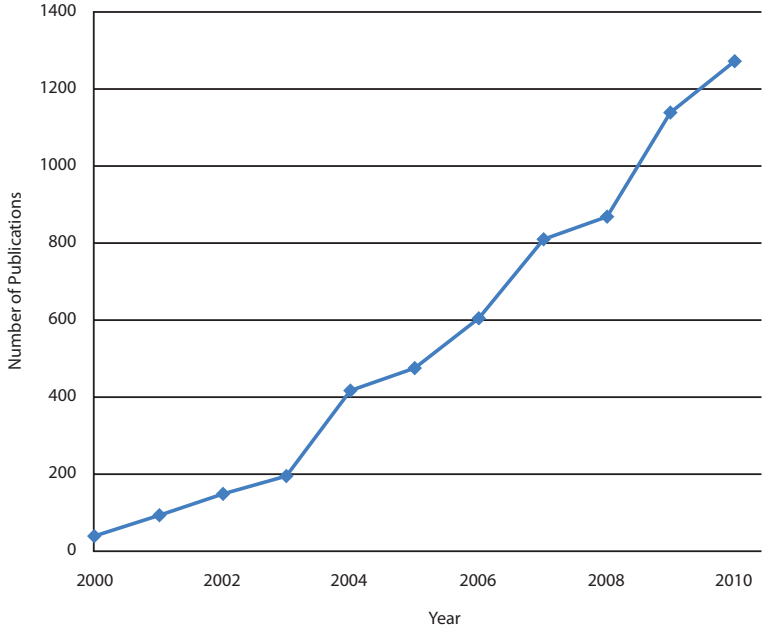


Figure 1 *Development of the number of publications within the microfluidics area in the PubMed database[3].*

To understand development on the commercial side, it is worth looking at the technology adoption model called "Gartner hype-cycle" [5], as shown in Figure 2 for microfluidics. This model has been used since the mid 1990s to describe the maturation of a new, disruptive technology in the conflicting fields of public expectations and technology deliverables, and is divided into different stages along the timeline.

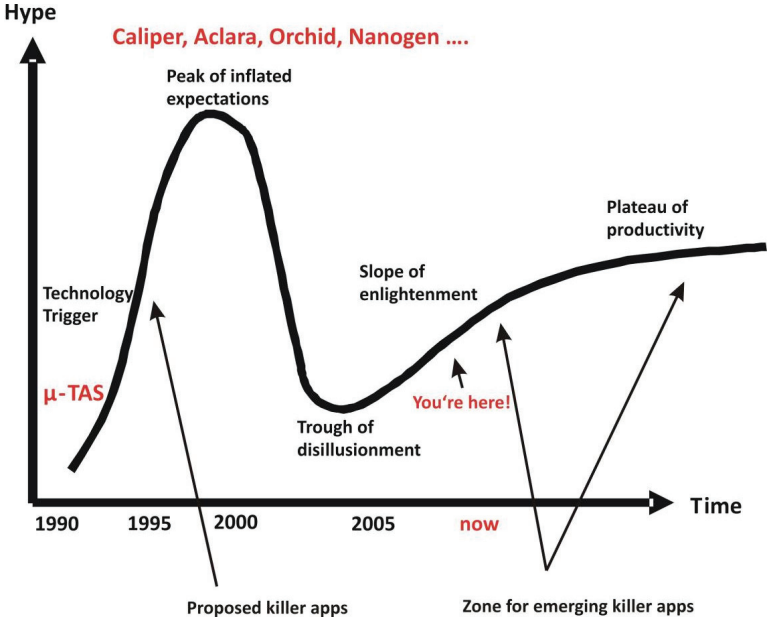


Figure 2 *The Gartner hype-cycle model for microfluidics.*

The first stage, the "technology trigger" is an event that launches a true innovation which captures the attention of a widespread audience beyond the inner scientific community. For microfluidics, Andreas Manz's conceptual µTAS paper in 1990[1] can be considered the technology trigger for microfluidics despite the influx of other papers that dealt with the handling of small volumes, *e.g.* micropumps or the visionary paper by Terry[6] about a fully integrated GC-system on a silicon wafer. The immanent reaction to this technology trigger, is the start of a technology hype which culminates in the "peak of inflated expectations". This phase happened in the years between 1995 and 2000 when practically every stakeholder expected microfluidics to revolutionise all aspects of the analytical and life sciences. Microfluidics made it into *Time* magazine and onto the cover of *Forbes*; pioneering companies such as Caliper, Aclara, Nanogen or Orchid Biocomputers were founded and went public, fuelling the hype even more with economic prospects until a "peak of inflated

expectations" was reached. However, the technology could not live up to these inflated expectations and suddenly, microfluidics lost much of its appeal. Commercial revenues did not grow as expected, market uptake of the "few" new instruments was much slower than anticipated, start-up companies folded and the revolution in the life sciences failed to materialise. This failure to deliver the promised result represents the typical next stage of the hype cycle which bottoms out in the "trough of disillusionment" which microfluidics hit around 2004. Then, something happened, which again is characteristic for almost any new technology, it came back (just think of web 2.0). Of course, there are reasons for such a comeback. In the first place, the original innovation was presented because there was a real need to solve a problem that could not be resolved by conventional means. In addition, since the technology trigger, the actual know-how about the technology increases and experience is gained. This allows a more realistic view of the technological and commercial possibilities. Suddenly, microfluidics is following the "slope of enlightenment", with less media coverage but with a much improved scientific, technological and manufacturing base. Although most of the pioneering companies have either disappeared or reduced their efforts in microfluidics, a second wave of companies is now employing microfluidics for their products. The main difference is that microfluidics technology is not highlighted. It has become a classical "enabling technology" which is used as a tool (like microelectronics or software engineering) to solve a specific application problem. The strength of microfluidics in this current state is the range of the applications it supports. Thus, instead of having a single or few killer applications, microfluidics is found in a large variety of very different applications. What these applications have in common is that they are either still in the development stage or in a comparatively early stage of their product life cycle, and most of them are not directed to end-user markets (private customers) but are sold in a business-to-business (B2B) configuration which includes the research and academic market. This usually means that the commercial volumes, and thus the revenues generated per product, are comparatively low, despite the fact that if added together the industry as a whole is already a decent size. To generate a true killer application, the industry will have to come up with a device or system which will make the transition from a B2B product to a consumer product. Only in this case can the volumes necessary to generate the much dreamed-of hundreds of millions product revenue be realised. This would happen in the last phase of the hype cycle, the "plateau of productivity". In this phase, the technology is widely accepted, has demonstrated viability and finds increasing use in a wider range of applications. The absolute height of this plateau, however, is determined by whether the technology

finds its killer application or if it remains in a market niche.
There are several drivers behind the current commercial development, practically all of them recognised as significant advantages in the pioneering age, but realised only very recently. Firstly, the fundamental method of mass transport by diffusion which governs many processes in chemistry and biology scales according to length[2]. This scaling allows us to develop systems *e.g.* for clinical diagnostics or analytical chemistry, where the overall time from the input of a sample to the analytical result can be reduced to minutes rather than the current hours or even days. Similar scaling advantages[7,8] can be found for other physical parameters, *e.g.* heat transport which is important for processes like the polymerase chain reaction (PCR). Secondly, the cost and the overall available volume of reagents in the Life Sciences is often a critical factor, *e.g.* in protein crystallisation or drug discovery. By reducing these volumes, not only can a cost reduction be achieved but often this represents the only way of handling and processing scarce material. Thirdly, many elements of biology, *e.g.* cells, blood vessels, bacteria *etc.* have a size which lies exactly in the range of micro technology, *i.e.* from 0.5 μm to about 100 μm. Fourthly, the very high geometrical accuracies of miniaturised systems together with the high surface-to-volume ratio (which also scales favourably with length^{-1}) makes the environment in which the fluids are contained extremely well controlled. This means that chemical reactions, for example, can be performed on a microfluidic device with a substantially higher yield than in conventional systems. Due to the small lateral dimensions, flows in such microstructures are nearly always laminar (low Reynolds number) which makes the flow extremely well controllable. Last but not least, miniaturisation offers the potential to automate many laborious laboratory processes that include many manual steps like pipetting, sample transfer *etc.*, reducing cost and time of the complete analytical process and minimising the risk of procedural error.

Advances in materials and fabrication technologies

The methods and materials used to create microfluidic devices are now significantly different compared to the situation 15 years ago. In the early years, microfabrication was strongly linked to methods and materials borrowed from the microelectronics industry, namely photolithography and wet chemical etching of silicon and glass. However, the progress made, especially in polymer microfabrication[10], has opened up routes to manufacturing which allows the generation of microfluidic devices outside of a cleanroom by a simple replication of a structure using elastomer casting[11,12,13], and the industrial high-volume production of such devices has improved by using injection moulding. Figure 3 shows

examples of the range of materials used in journal articles published in "Lab-on-a-chip" between September 2009 and September 2010. More than two-thirds of the articles use devices with a polymer as the main material, while silicon and glass account for less than 20%.

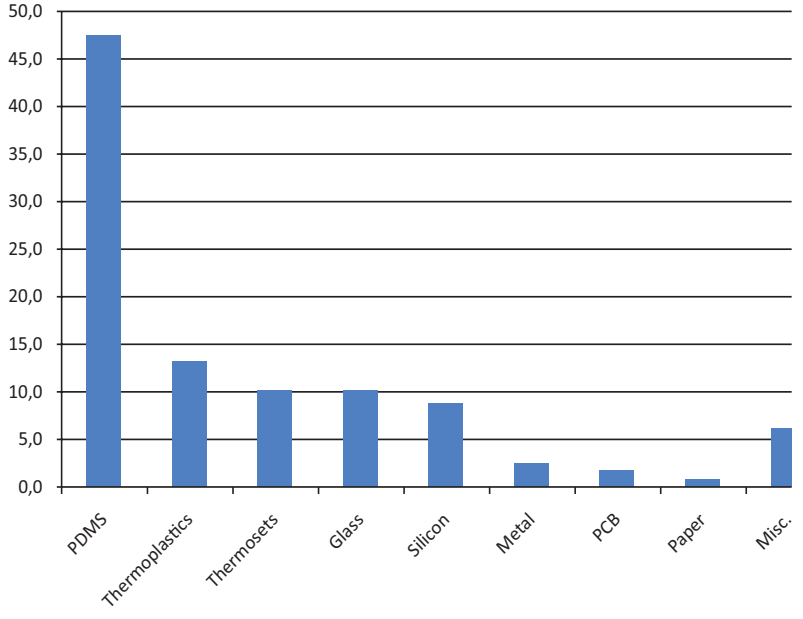


Figure 3 *Relative frequency in percentage of materials used in Lab-on-a-Chip publications between September 2009 and September 2010.*

In Figure 4, the technology chain necessary for manufacturing microfluidic devices is shown with two main branches, the upper branch describing the methods for silicon and glass-based devices, the lower branch for polymer-based devices.

The manufacturing process in both cases starts with the design of the device. Beyond the application-driven aspects, input from the complete manufacturing chain is necessary to achieve what is called design-to-manufacture, *i.e.* a device design which allows the manufacturing at a given cost. For glass- and silicon-based devices, the conventional lithography-based approach involves a mask and the subsequent transfer of the mask pattern using lithography and subsequent wet (or dry) chemical etching. Alternatively, for larger structural dimensions, the pattern can be transferred using sand or powder blasting[14] which allows higher etch rates (typically >20 µm min^{-1}) and aspect ratios of about 1.5–2, compared with etch rates of typically less than 10 µm min^{-1} and, due to the isotropic nature of the wet etch, aspect ratios of less than 0.5 in case of the conventional wet etching technology. A special case is the

photostructuring of glass, either by using a special photosensitive glass that develops upon exposure by UV light microcrystalline regions which then can be etched out using hydrofluoric acid (HF)[15] or by exposing the glass to a femtosecond laser beam that induces material changes which again change the etch selectivity for a subsequent HF etch[16].

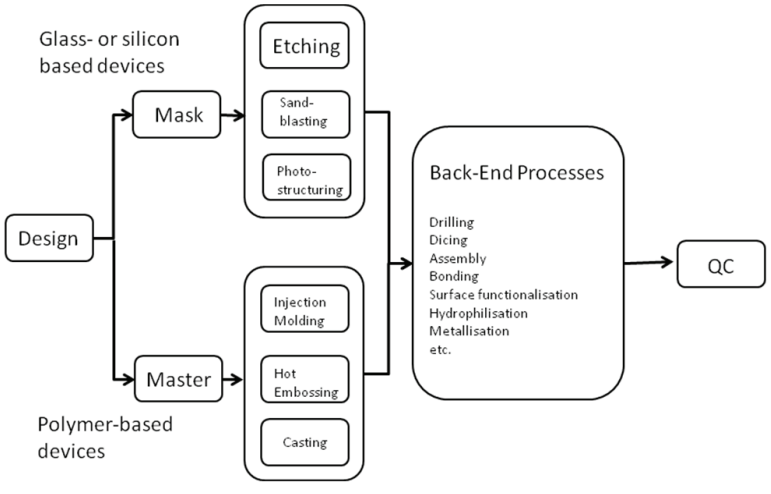


Figure 4 *Process chain for the manufacturing of microfluidic devices in glass or silicon (upper part) and polymers (lower part).*

In the case of polymer replication methods like injection moulding and hot embossing, the device design has to be translated into a replication master (often with some verbal unspecificity referred to as "replication tool", "mould" or "mould insert"). Although the requirements for such a master structure differ with respect to the physical parameters of the chosen replication method (*e.g.* force and temperature), four basic statements can be made: (a) the geometrical replication result can only be as good (or as bad) as the geometrical accuracy of the master; (b) for the ability to separate mould and moulded part (demoulding step), no short cuts in the structure itself can be allowed; (c) the surface roughness of the master should be as low as possible (ideally peak-valley values of below 100 nm); and (d) a suitable interface chemistry between the master and substrate has to be chosen. In order to generate the master structure, nearly all microfabrication methods are suitable. The correct selection of the master fabrication technology is a crucial step in the product development of commercial microfluidic devices, especially as there is no generic recipe for this selection. Table 1 lists the most common master fabrication methods with their properties. For commercial applications, the most suitable methods are precision-machined masters made out of steel for structural dimensions down to about 30–50 μm or, for smaller structures,

Table 1 *Overview on main master fabrication methods*

Microfabrication technology	Choice of geometry	Minimum feature size	Structural height	Total surface area	Aspect ratio	Lifetime	Cost	Commercial availability
Wet silicon etching	–	+	0	+	–	0	+	+
Dry silicon etching	+	++	+	+	++	–	0	++
Lithography & electroplating	0	++	+	++	+	+	0	0
Laser ablation & electroplating	+	+	+	++	0	+	–	–
Femtosecond laser machining	+	+	0	–	0	+	–	–
Mechanical (ultra) precision micromachining	++	0	++	++	+	++	–	+
LIGA	+	++	+	–	++	+	–	–
Thermoset structuring	0	++	+	++	+	–	0	–
µ-EDM	–	0	+	++	+	++	–	–

nickel masters which are generated by electroplating of photoresist or silicon. For larger structures, micro-electrode discharge machining (μ-EDM), which is one of the most common methods for stainless steel tooling in the macroworld, becomes possible. Both methods combine long master lifetimes with good geometrical definition at reasonable cost and availability. The LIGA (Lithographie, Abformung, Galvanoformung) process[17] generates masters with the highest precision and best surface roughness available, however, the manufacturing process is complex, expensive and time consuming. On the other hand, masters consisting of silicon can be made quickly and at low cost. Due to the brittleness of the material they can mainly be used for casting and hot embossing. A recent development is the use of polymers (*e.g.* fully cured SU-8 photoresist) as a material for replication masters[18]. The master's lifetime in this case is limited typically to a few tens to hundreds of replications at moderately complex designs and low aspect ratios. For more complex geometries, namely when comparatively large structural dimensions (mm-sized features) have to be combined with small features, hybrid tooling such as the combination of precision machining for the larger features and lithography or laser ablation for the finer features can offer a solution. Figure 5 shows an example of a precision machined stainless steel master structure.

Figure 5 *Mould insert for a standardised injection moulding tool (microscopy slide size) made with precision mechanical machining.*

For the high volume manufacturing of disposable microfluidic devices, high-precision injection moulding has established itself as the method of choice. As it is by far the most widespread fabrication process for polymers in the macroworld, it is not surprising that the first application of this production technology for microfluidic components was published more than 10 years ago[19]. Due to the comparatively high demands in equipment and process, it is comparatively seldom used in academic compared to industrial use. For the commercial success of microfluidics, it will, nevertheless, play a crucial role.

One of the constraining factors in injection moulding is the high specification for the so-called injection moulding tool. It has to perform very precise mechanical motions under high temperature variations and forces. Figure 6 shows an example of such an injection moulding tool for microtiter-plate sized devices. The mechanical complexity of such

an injection moulding tool is apparent. One of the strategies to reduce the development cost of microfluidics devices is the use of so-called family tools that use exchangeable mould inserts and can thus be used for moulding different device designs. The moulding process has various steps. The polymer material is fed as pre-dried granules into the hopper. In the heated barrel, a screw transports the material towards the injection port of the moulding tool. During this transport, the polymer melts and reaches the tool in liquid form with a melt temperature of the order of 200–350ºC depending on the polymer. It is now injected under high pressure (typically between 600 and 1000 bars) into the mould which contains the microstructured mould insert. For microstructure replication, it has to be evacuated to achieve good filling of the mould and to prevent the formation of air pockets. Depending on the surface-to-volume ratio of the structure, the mould can be kept at temperatures below the solidification temperature of the polymer (typically between 60 and 120ºC, the so-called "cold cavity process") or, in case of small injection volumes and high aspect ratio structures, it has to be kept at temperatures above the so-called glass-transition temperature T_g and cooled together with the melt. The need for this so-called "variotherm process" drastically increases the cycle time. Therefore in commercial applications, the development of the microstructure has as one goal the mouldability using a cold cavity process. Typical cycle times for a cold-cavity process are of the order of 30 s to 2 minutes, a variotherm process can take up to 5 minutes. After opening of the mould, the moulded part will be ejected from the mould. Normally, remains of the material from the injection port (so-called "sprue") will still be connected to the part which has to be removed, either mechanically by cutting, sawing, breaking off or by using a laser.

Figure 6 *Injection moulding tool for microfluidic devices with the size of a microtiter plate. Note the mechanical complexity.*

Figure 7 shows an example of a multi-level structure injection moulded from a mechanically machined mould insert. The great advantages of injection moulding are the ability to form three-dimensional objects which, in the case of microfluidic devices, means *e.g.* the integration of fluidic interconnects[20] (see Figure 9) or through-holes. Furthermore, the ejected part does not normally need additional process steps, thus reducing the

need for mechanical back-end processes (see below). At this point in manufacturing, one has a microstructured glass, silicon or polymer part. For prototyping or low volume manufacturing, most of the cost is concentrated in this part[21]. However, for higher volumes, the majority of production cost lies in the subsequent back-end processing steps. These can be roughly divided into two groups:

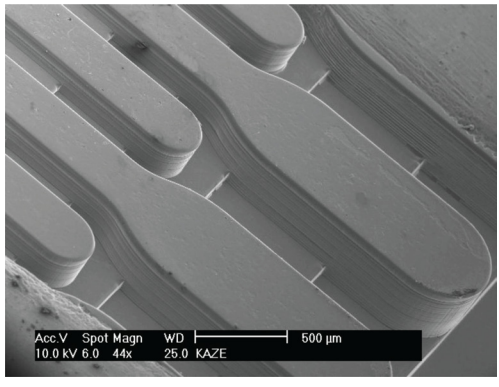


Figure 7 *Multi-level microfluidic structure injection moulded from a mechanically machined mould insert.*

1. *Physical processes:* In this category mechanical process, steps include dicing the microfluidic device out of the wafer, the drilling of access holes, the bonding of a cover lid in order to close the channels, the assembly of the device in case of the integration of sensors, gaskets, membranes, septa or other components and similar physical processes. A special process is the integration of electrodes onto the polymer device. This can be realised using thin-film processes like thermal evaporation or sputtering[22] or thick-film processes like screen-printing[23]. Recently, ink-jet printing of conductive inks[24] has received increasing attention due to its cost and the ease of integration of this process into the overall production line.
2. *Chemical processes:* These processes are related to the surface chemistry of the device. Frequently, this surface chemistry, especially the hydrophilicity/hydrophobicity of the device, has to be tuned for a specific device[25-27]. As most thermoplastic polymers have a fairly hydrophobic surface (contact angle typically 80–90˚), the contact angle has to be decreased in order to obtain devices that fill by capillary action. This can be realised using a plasma treatment, UV light or by flush-coating the enclosed microchannel. Other surface modification steps include the local binding of (bio-) molecules to the surface (*e.g.* for array-based assays) or a local deposition of reagents, *e.g.* lyophilised or in biostabilised form. This form of chemical patterning is normally realised using a spotting tool or inkjet-like printing.

In an industrial setting, the manufacturing chain is finished by a quality control process, usually a combination of optical inspection with a functional test of a subsample which is selected by statistical process control (SPC) methods and the subsequent packaging of the device in a suitable form, *e.g.* pouches or sealed foil packs.

It is important to note here that the biggest potential for cost-saving lies in using a clever device design which can reduce or simplify the back-end processing steps[12].

The development of integrated microfluidic devices

Strategies for complex microfluidics device development

One of the most important advances in recent years has been the ability to transfer complex analytical or diagnostic processes onto a single microfluidics device. For the development of such an integrated device, a two-prong approach has proven to be useful. On one hand, a holistic top-down approach from the system level is necessary in order to ensure the inclusion of all necessary functions as well as the definition of all interfaces (fluidic, mechanic, optical, *etc.*). A flow-diagram of all process steps performed on the device can then be translated into individual functional modules. The second line of approach is then a development, *e.g.* by simulation[28] and subsequent prototyping, of the individual module, *e.g.* a DNA extraction chamber, a mixing structure for the lysis buffer, *etc.*, where the individual functions can be validated before integration. There is one significant difference between microfluidics and other engineering disciplines, especially mechanical or electrical engineering. In these fields, the development of individual modules tends to be simpler due to the fact that the mutual interactions of the individual modules are more limited and often calculable with simple restraints, allowing the assembly of module libraries which can simply be transferred from one development to another. In microelectronics, an operation amplifier or a storage capacitor will behave (almost) identically regardless of the overall system layout. In microfluidics, however, the performance of a single module is often largely dependent on the overall system layout. A typical example would be the parameter of flow speed in a microfluidic module, *e.g.* a simple T-shaped microchannel. This flow speed can be easily calculated given the dimension of the various arms of the channel. However, as the flow speed depends (amongst other things) on the back-pressure, the different arms of the T are experiencing, the flow distribution changes depending on the back pressure generated by preceding or succeeding modules. If this happens in a time series, the functional description of such a simple module can become quite difficult. It is therefore emerging as best practice to combine the theoretical (or modelling) approach with some experimental data from module prototypes.

Figure 8 shows typical process steps that have to be realised during a development of such an integrated microfluidic device.

In the first step, the sample has to be brought onto the device through

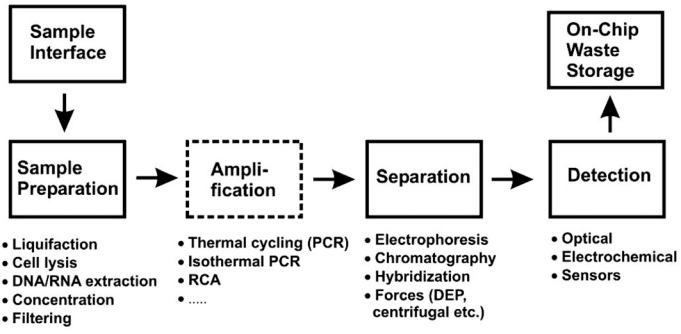


Figure 8 *Schematic diagram of the typical process steps involved in a bio-analytical or diagnostic process flow in a microfluidic device.*

some interface[29]. As the type of sample can be very different (*e.g.* biopsy, swab, sputum, blood, *etc.*), this interface has to be adapted. On the one hand, an efficient sample transfer to the device must be ensured, on the other hand, the absence of any contamination of the sample or infection risk of the operator must be avoided. These "world-to-chip" interfaces are often an over-looked but important item during the development of microfluidic systems. Increasingly, the use of existing standards from the targeted application area (*e.g.* Luer-Lok compatible interfaces in clinical diagnostics) is being established, however, with disadvantages mainly in terms of size. For this reason, we have developed a similar press-fit interface with a reduced footprint (called "Mini-Luer"), allowing up to 32 fluidic ports on a device the size of a microscopy slide. Figure 9 shows for comparison, microfluidic chips with (from left to right) tube connectors, Mini-Luer and full-size Luer connectors.

The next step, the various sample preparation processes such as liquefaction of the sample, the lysis of cells, extraction of DNA/RNA, the sample concentration *etc.*, have so far been typically been carried out off-chip due to their complexity and the different nature of the samples. Incorporating these steps onto the device represents the biggest challenge[30] mainly due to the fact that several media (wash buffer, carrier buffer, beads, lysing

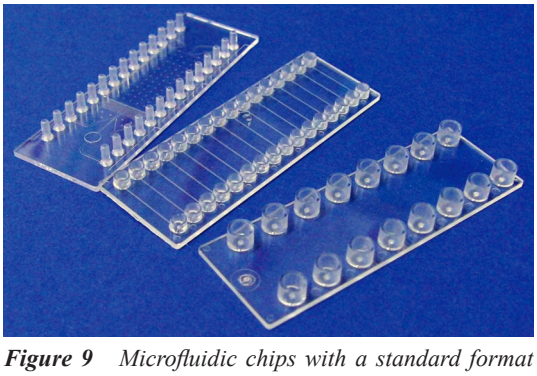


Figure 9 *Microfluidic chips with a standard format (microscopy slide) and different fluidic interfaces. From left to right tube connectors, Mini-Luer and full-size Luer connectors.*

agents *etc.*) have to be handled sequentially as well as in parallel, which all require inter-faces and plumbing in very restricted device areas. Furthermore, many of these steps have to be carried out with a high precision in terms of volume, times or sequence which in specialised (and often costly) laboratory equipment is much less difficult to achieve. It is therefore a specific requirement in the development of miniaturised assays that the assay should be as robust as possible in terms of process steps, volumetry and timing in order to be carried out on-chip. The next process step, usually in devices using molecular biology methods, involves an amplification of the target molecules. Using methods like conventional[31] or isothermal[32] PCR, rolling circle amplification[33] (RCA) to increase the number of target molecules achieves better detection selectivity and sensitivity. This amplification step is then frequently followed by a separation step like electrophoresis, chromatography (which up to now has not been not well developed on-chip), the use of capture probes (*e.g.* DNA arrays[34]) or other filtration mechanisms in order to isolate the desired component spatiotemporally or to remove unwanted components from the mixture.

The final analytical step comprises the detection of the analyte of interest. For many larger, lab-based systems, optical detection methods[35] like laser-induced fluorescence still act as a benchmark with respect to sensitivity. However, for portable systems, electrochemical analysis methods[36] or various other sensor methods [*e.g.* surface acoustic waves[37] (SAW), quartz crystal microbalance[38] (QCM), thermal measurements] are becoming increasingly interesting. It should be noted that all the preceding process steps have to be matched to the selected detection method in order to generate the best results.

A minor but, nevertheless, important design step of an integrated device in diagnostics is the layout of a waste container system in order to retain all liquids used in the process on-chip. This is often necessary to avoid the contamination risk of the instrument and to prevent carry-over from one measurement to the next. The required volume of such waste reservoirs can be critical, frequently stressing the limited real estate on the chip.

Once these individual functions have been verified on-chip, a stepwise integration into a single device then can take place. This stepwise approach also simplifies the search for, and correction of, possible errors observed in the performance of the device.

Development example

In order to elucidate the development and manufacturing processes described above, the stepwise realisation of an integrated device for the simultaneous detection of eight different pathogens is now described.

It starts with the selection and development of individual functional modules and culminates in the complete device integration.

Functional modules

The first example of such a functional module is a chip that contains magnetic beads for DNA extraction. The chip (the size of a microscopy slide) contains two rhombic chambers each with a volume of 120 µL. These chambers are either pre-loaded with coated magnetic beads or can be loaded after assembly, as shown in Figure 10. The sample is introduced into the chamber together with lysis buffer and incubated for 5 minutes. This is followed by three subsequent washing steps with wash buffer; after each washing step, the magnetic beads are held at one end of the chamber using a magnet to concentrate the beads at the desired location. After the final wash, the buffer is replaced by an elution buffer in which the DNA bound on the magnetic beads is released. After collecting the eluate DNA, it can be then transferred to an amplification module. Figure 11 shows the amplification results in a 36 cycle PCR chip of a

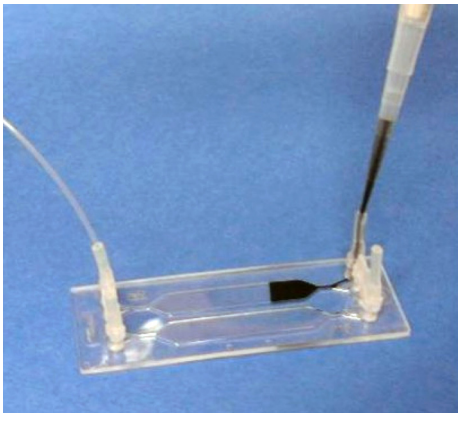


Figure 10 *Chip for DNA extraction using magnetic beads. The beads together with lysis buffer are pipetted into the chip.*

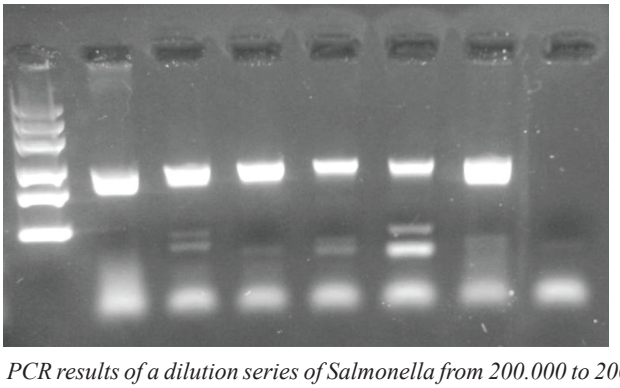


Figure 11 *PCR results of a dilution series of Salmonella from 200.000 to 200 bacteria from DNA extracted with the chip shown in Figure 10. The lanes contain from left to right Lane 1, mass ruler. Lane 2, 200.000 bacteria. Lane 3, 20.000 bacteria. Lane 4, 2.000 bacteria. Lane 5, 200 bacteria. Lane 6, positive control for 200 bacteria in a conventional PCR cycler. Lane 7, negative control.*

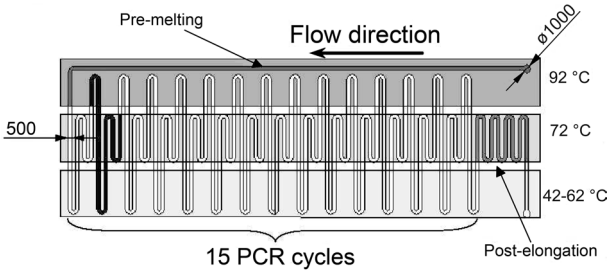


Figure 12 *Principle of chip-based continuous-flow PCR. The sample is pumped over three stationary temperature zones, thus eliminating the need for thermocycling.*

dilution series of Salmonella as a model organism for the pathogens. The specific fragment size of the PCR product was 263 bp.

The next module is an amplification module utilising the principle of continuous-flow PCR[39,40] which is a unique technology used in microfluidic devices. This principle is especially suited for long-term, decentralised monitoring purposes as it operates with stationary temperature zones instead of conventional thermocycling, thus greatly reducing the energy requirements. At the same time, the analysis speed is improved while very low sample volumes are required. Furthermore, this principle lends itself for continuous monitoring *e.g.* in the case of air-borne pathogen monitoring with the sample being continuously taken from an air sampler. Figure 12 shows the principal layout of such a continuous-flow PCR chip with the three temperature zones required to perform the PCR process; Figure 13 the actual chip with 36 PCR cycles as used above, injection moulded in polycarbonate (PC).

The final step in the analytical process is the detection of the relevant sample. Up to now in most cases, optical detection methods, namely fluorescence, are used because of their high sensitivity as well as the large number of protocols and dyes available.

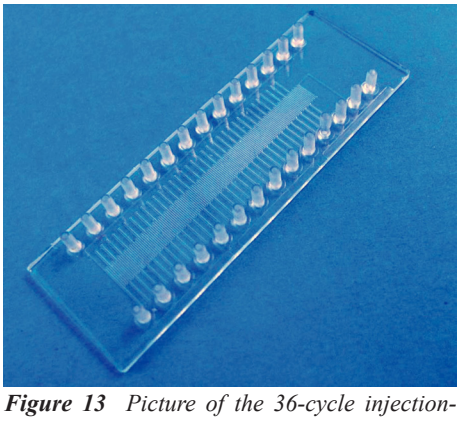
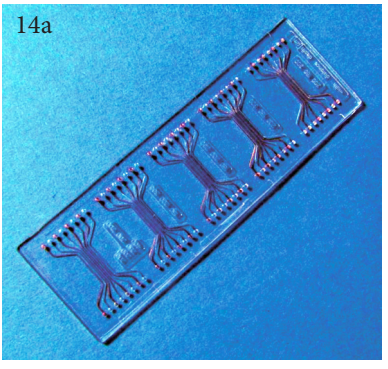


Figure 13 *Picture of the 36-cycle injection-moulded continuous-flow PCR chip.*

An example of a chip module made for the fluorescence end-point detection of a qPCR process is shown in Figure 14a and a subsequent fluorescence image of the detection area in Figure 14b with the measurement of qPCR products in Figure 14c. As in the final device, an eight-plex detection was targeted, the spacing of the microchannels after qPCR had



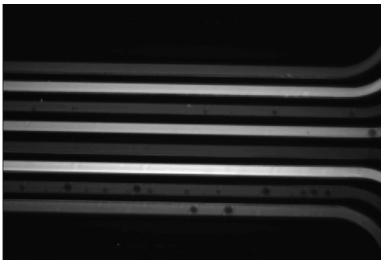
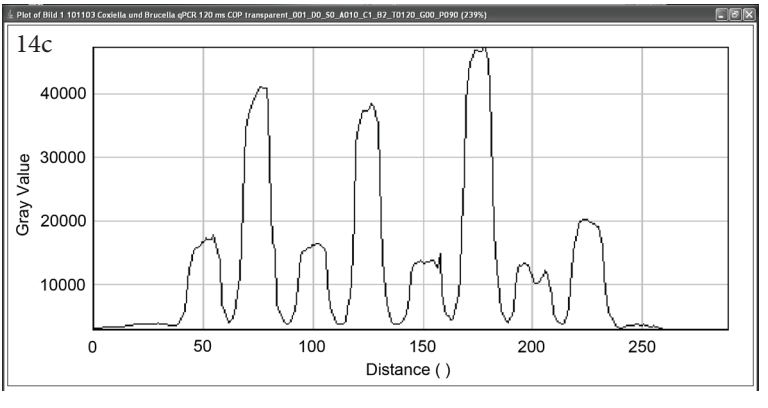


Figure 14 (a) *Channel array chip for the optimization of the detection zone geometry.* ***(b)*** *Fluorescence image of PCR products in the detection zone.* ***(c)*** *Fluorescence data of PCR products in detection zone.*



to be very narrow in order to have all channels within the field of vision of the detection system in order to avoid the need for optical scanning. This led to 200 µm wide, 300 µm deep microchannels with a spacing of only 150 µm which poses a significant challenge in the leak-tight bonding of the device with a thin cover foil. As can be seen from the fluorescence image, no cross-reaction due to incomplete bonding is visible.

Integrated devices

After validating the modules above, an integrated device for the detection of eight different pathogens from a single sample was developed. The chip with the footprint of an SBS titer plate is shown in Figure 15 which consist of a single injection moulded polymer part and which contains microfluidic structures on both the top and on the bottom. The sample is introduced in the upper right hand corner through a Luer connector. Then, a bead-based DNA extraction and concentration takes place. The

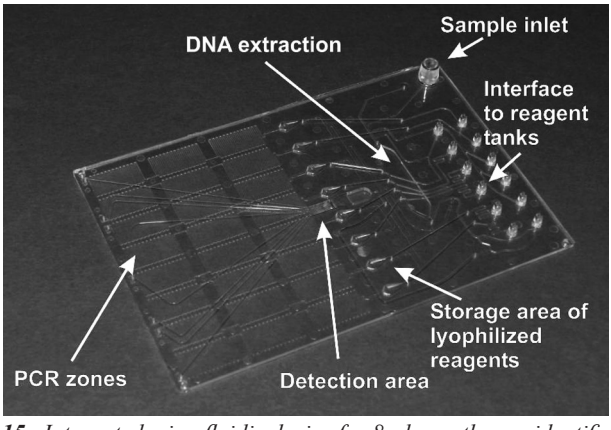


Figure 15 *Integrated microfluidic device for 8-plex pathogen identification.*

sample is subsequently divided in eight aliquots which flow through the storage area where the lyophilised PCR master mixes are stored. After liquefaction of the PCR mixes through the sample, the continuous-flow PCR takes place. The sample is then transported through microchannels on the top side of the chip to the detection area, where the fluorescence detection takes place before the samples are sent to waste.

With this device, experiments for the simultaneous detection of *Brucella melitensis, Burkholderia mallei, Burkholderia pseudomallei, Coxiella burnetii, Francisella tularensis, Yersinia pestis* and *orthopox* virus are currently under way to determine the performance of the device. An overall analysis time including all sample preparation steps as well as PCR of 40–45 minutes is targeted, which is comparable to the fastest commercially available, but significantly more complex and expensive, systems.

Applications

As an enabling technology, microfluidics has found its way into a large number of different applications.

Application examples

Historically, the initial showcase and first commercial application was capillary electrophoresis (CE)[41]. In this technique, a complex mix of molecules is separated according to their electrophoretic mobility which is determined by factors such as relative charge and size of the molecule. The separation takes place under the influence of an electrical field which is applied along the microchannel, with the species with the highest mobility arriving in the detection zone first, and is then followed by the other species in order of decreasing mobility. Transferring these techniques

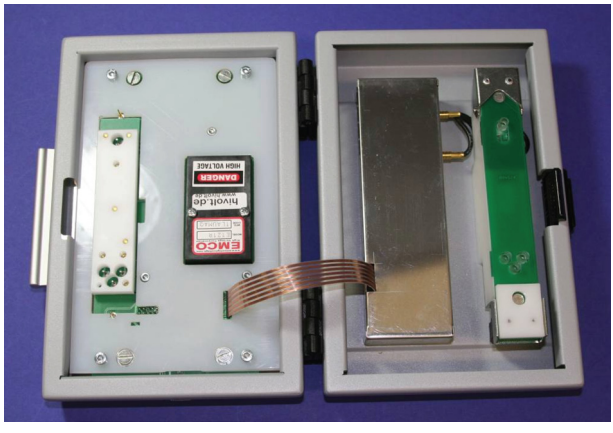


Figure 16 *Instrument for chip-based capillary electrophoresis* ***(a)*** *and associated microfluidic chip* ***(b)***.

onto a microfluidic chip allows higher field strengths and fast dissipation of the generated Joule heating, leading to extremely fast separations. Several analytical systems using this technique are commercially available, *e.g.* from Caliper, Bio-Rad or Shimadzu. Figure 16a shows as an example a compact chip-based instrument from microfluidic ChipShop in its opened state, the left side contains the high voltage section for the separation voltage and the electrodes which contact the fluidic reservoirs, the right side the detection electronics and the chip holder. The size of the instrument is only about 19 cm × 12 cm × 8 cm, making such a system applicable for field use in places such as food processing plants or water works. The separation takes place in disposable polymer chips shown in Figure 16b. The microfluidic channels have a cross-section of 50 × 50 µm, the complete chip has a length of 95 mm and can be manufactured in high volumes using injection moulding.

A second commercial applications is microreaction technology[42] for the production of fine chemicals. In this case, the superior mixing and reaction control properties of microfluidic systems are utilised to perform

chemical reactions or syntheses at much better yields and better selectivity than in conventional systems. By reducing the diffusion length, chemical reactions can take place much faster. At the same time, in combination with the increased surface-to-volume ratio, heat generated by the chemical reactions can be removed from the system at a much higher rate, thus allowing such reactions to take place in process conditions that would not be possible in conventional large-scale systems. Several companies such as Ehrfeld Mikrotechnik BTS, mikroglas chemtech or Corning have commercial systems on the market.

One area which has recently started to massively profit from the benefits of microfluidics is cell biology[43-45]. As cells have typical dimensions of the order of 5–20 μm, this is an ideal for the size range of typical microfluidic structures. The applications range from the observation of the physical and biological behaviour of single cells in different culturing media, chemotaxis experiments to observations of growth patterns, the guidance of growth, *e.g.* of neurons to a complex assembly of different cell types in order to develop artificial organ-like cell assemblies on a chip[46]. This can potentially be of great importance in the drug research[47], as the biological behaviour of cells in conventional cell cultures is usually very different from an *in-vivo* situation. On a chip, however, due to the strict control of the environment, cells can be assembled in conditions which are close to the in-vivo situation. One can therefore study the reaction of cells and cell-assemblies *e.g.* in the presence of certain drugs and gain information on relevant drug dosages or toxic effects. Already, several commercial systems for studying cells in microfluidic environments are available from companies such as Fluidigm, Cellix, Bell Brook Laboratories or CellAsic.

The biggest predicted market for microfluidic devices and systems, however, comes from the diagnostic market[48,49], especially molecular diagnostics. Microfluidics and miniaturisation technologies have a crucial enabling role for new product development in this field[50], as the required integration density, portability and speed for such applications can only be realised in miniaturised solutions. Furthermore, many of the diagnostic procedures require the integration of methods of molecular biology like DNA extraction or PCR which only in their microfluidics-based protocols can be performed outside a specialised laboratory. Figure 17 shows such a highly integrated chip for the detection of breast cancer markers[51]. Similar requirements exist for the identification of pathogens in biothreat detection[52], where very small amounts of viruses or bacteria have to be identified. A large number of commercial activities from companies such as Abbott, Idaho Technologies, Zygem, Alere and many others (for a company list see ref. 53)

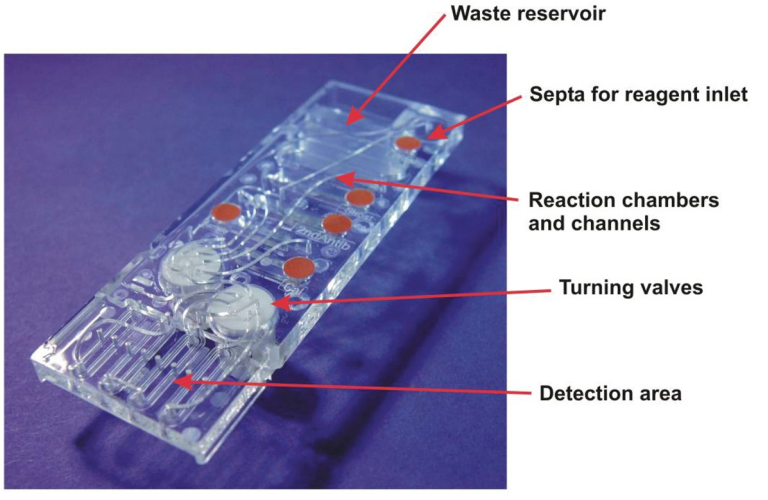


Figure 17 *Integrated microfluidic platform chip for the detection of breast cancer markers (realised within the EU-FP6 project "SmartHEALTH", No. 016817).*

Future trends

Given the enormous growth in technologies and applications, no simple trends can easily be identified. However, several discussions have taken place within the scientific community that reflect the directions in which the field is likely to develop.

a) Standards

It is obvious that, at least in theory, standardisation helps in increasing production volumes and thus, through learning curve and economy-of-scale effects, leads to reduced manufacturing cost. This in turn increases market sizes and competitiveness of the technology[54]. The microelectronics industry is an example which is often quoted in this context. However, there are significant differences in the methods, materials, applications and business models between the microfluidic and microelectronics industry. There are several areas in which a certain degree of standardisation even for the broad range of microfluidic applications can be seen as useful. These areas comprise for example, the outer dimensions of a microfluidic device where existing quasi-standards like microscopy-slide or SBS titer plate exist and where the utilisation of these standards facilitate the integration of microfluidic devices into the daily laboratory workflow. A second area where a debate is ongoing concerns microfluidic interconnects. In this area, the requirements are more divergent with respect to performance parameters such as dead volume, allowable pressure or footprint. Again, using existing standards from the target markets is an obvious starting point. Other discussions

concern measurement characteristics and materials but these are in very early stages of their development.

b) Instrumentation

Research and development in the early years concentrated on the realisation of the microfluidic devices, using macroscopic peripheral instrumentation for fluid control, manipulation and detection. This has often led to a description of a "chip-in-a-lab" instead of "lab-on-a-chip". The ability to integrate more functionalities onto the microfluidic device, the advent of semiconductor optics (LEDs, lasers and CCDs) as well as sensor technologies for the detection have moved the technology closer to the initial expectations of systems with a small footprint or even handheld, portable instruments. Applications such as point-of-care diagnostics or continuous pathogen monitoring support such efforts in reducing the overall instrumentation load as well as the need for simple and robust diagnostic or analytical instrumentation in low resource settings[55]. This trend is ongoing and it can be expected to bear fruit over the coming years.

c) Ease of use

Another beneficial feature of microfluidic systems that only recently has become realised but which plays an important role commercially is the potential of a very simple operation of the system, replacing manual sample handling steps and expensive instrumentation. This feature allows analytical instrumentation to be used by only moderately trained staff in the field which is especially important for applications such as forensics, on-site food analysis or veterinary diagnostics. It is also an important argument with respect to quality assurance, as the reduction of manual process steps also reduces the number of possible human-induced errors in the analytical process. For many cost sensitive applications, this is also an important driver for the introduction of new technologies.

Conclusions

Microfluidics technology has made enormous progress in the last 15 years and has proven that it is viable in the scientific as well as the commercial arena. Although the commercial development did not happen as fast as many people predicted 15 years ago, it is evident that microfluidics has turned into a crucial enabling technology for almost any product development in the life sciences and the list of microfluidics-enabled products is steadily rising. The big killer application is still missing, but comparison with the market uptake of other high-tech applications shows that the current time-line is nothing extraordinary. The range of applications is extremely broad and even if it has not revolutionised

the life sciences as many had hoped, it has and is still changing many established practices in these disciplines. It is worth to looking at a quote from the 1996 Science Progress paper[2]:"Admittedly, it is a long way to the neat little Tricorder. But with more than 20 research teams around the world involved and a biannual conference, entitled μ-TAS, this exciting new research field already displays rapid progress". Then note that the X-Prise foundation is currently preparing a 10 million USD Tricorder X-Prise challenge[56]. So fast, science-fiction can become a reality. But nobody has invented the warp-drive yet…

Acknowledgements

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