Lab on a Chip

Miniaturisation for chemistry, biology & bioengineering

www.rsc.org/loc

Volume 7 | Number 9 | September 2007 | Pages 1081–1220

ISSN 1473-0197

Microfluidic platforms for lab-on-a-chip applications

Stefan Haeberle[a] and Roland Zengerle[ab]

Received 26th April 2007, Accepted 25th June 2007
First published as an Advance Article on the web 27th July 2007
DOI: 10.1039/b706364b

We review microfluidic platforms that enable the miniaturization, integration and automation of biochemical assays. Nowadays nearly an unmanageable variety of alternative approaches exists that can do this in principle. Here we focus on those kinds of platforms only that allow performance of a set of microfluidic functions—defined as microfluidic unit operations—which can be easily combined within a well defined and consistent fabrication technology to implement application specific biochemical assays in an easy, flexible and ideally monolithically way. The microfluidic platforms discussed in the following are capillary test strips, also known as lateral flow assays, the ''microfluidic large scale integration'' approach, centrifugal microfluidics, the electrokinetic platform, pressure driven droplet based microfluidics, electrowetting based microfluidics, SAW driven microfluidics and, last but not least, ''free scalable non-contact dispensing''. The microfluidic unit operations discussed within those platforms are fluid transport, metering, mixing, switching, incubation, separation, droplet formation, droplet splitting, nL and pL dispensing, and detection.

Introduction: the need for microfluidic platforms

The impact of microfluidic technologies in the academic world has dramatically increased during the last years. This is quite amazing since microfluidics is no product that a consumer wants to buy. Microfluidics should be merely considered as a toolbox, which is needed to develop innovative new products in the life sciences. As a consequence, the most important customer for microfluidic know-how and technologies is the research community itself, developing new products and solutions in such different application areas as the biotechnology, diagnostics, medical or pharmaceutical industries.

The history of microfluidics dates back to the early 1950s, when efforts to dispense small amounts of liquids in the nano- and subnanolitre ranges were made for providing the basics of today's ink-jet technology.[1] In terms of fluid propulsion within microchannels of sub-millimetre cross section, the year 1979 set a milestone when a miniaturized gas chromatograph (GC) was realized on a silicon wafer.[2] The first high-pressure liquid chromatography (HPLC) column device, fabricated using Si–Pyrex technology, was published by Manz *et al*.[3] By the end of the 1980s the first micro-valves[4] and micro-pumps[5,6] based on silicon micro-machining had also been presented. Within the following years several silicon based analysis systems have been presented.[7,8] All these examples represent microfluidic systems since they enable the precise control of the decreasing fluid volumes on the one hand and the miniaturization of the size of a fluid handling system on the other hand.

[a]*HSG-IMIT—Institute for Micromachining and Information Technology, Wilhelm-Schickard-Straße 10, 78052 Villingen-Schwenningen, Germany*
[b]*Laboratory for MEMS Applications, Department of Microsystems Engineering (IMTEK), University of Freiburg, Georges-Koehler-Allee 106, 79110 Freiburg, Germany. E-mail: stefan.haeberle@hsg-imit.de; Fax: +49 761 203 7539; Tel: +49 761 203 7476*

Following these pioneer works, thousands of researchers spent a lot of time in developing new microfluidic components for fluid transport, fluid metering, fluid mixing, valving, or concentration and separation of molecules within miniaturized quantities of fluids within the last two decades. Today, many different types of micro-pumps have been described in publications,[9–12] many different types of mixers[13,14] and many different types of microvalves[15] are known and nearly no standards are defined in terms of interconnections, *etc*. It seems to be the right time to raise the question whether we really need more of those components? In our opinion, for exploring the huge potential of different applications in the lab-on-a-chip field, a component based microfluidic approach is much too slow and the R&D effort much too expensive. In addition, the best performance you can get out of such a *''component oriented solution''* will be far behind what you can get in an *''integrated system approach''* or in other words a *''microfluidic platform approach''*. Therefore, we think that the described practice of assembling discrete components like valves and pumps, at least in the field of lab-on-a-chip applications, belongs to the past and we do not expect that it will continue in the future. In our view the research community really needs validated and easy to operate microfluidic platforms. These offer an adequate number of *microfluidic unit operations* which can be easily combined to build application specific microfluidic systems. In addition, those systems should be producible in a standardized cost efficient technology.

Before we point out the power of the microfluidic platform concept further, we describe the opposite of it: an example of an application specific integrated system, representing a unique engineering solution to a unique technical problem. The ''electronic fountain pen''[16] is a good example of such a discrete microfluidic solution. It can be regarded as the first fully functional, highly integrated, miniaturized and self-sustaining microdosage system of its kind operating under

real world conditions. The main components have been a liquid level sensor, a microvalve and a bubble and particle tolerant fluidic system. The pen has been optimized with respect to minimum energy consumption. It contains a programmable ASIC and is powered by two standard watch batteries, ensuring operation over a period of 2 years under standard conditions. The electronic fountain pen perfectly fulfils the requirements for its specific application. For any other application in the field of micro-dosage, or more generally in the field of microfluidics, however, the specific know-how from developing such a system is only of very limited value and every development of this kind always starts from scratch again. This causes significant costs and time at a high economic risk. Although we expect that this kind of development makes sense for a few selected applications in diverse fields of applications, in the future also, it is quite clear that this approach will not succeed for lab-on-a-chip systems.

What is needed for these applications, in contrast to unique solutions, is microfluidic platforms. Very similar to the ASIC industry in microelectronics, which provides validated elements and processes to make electronic circuitries, a dedicated microfluidic platform comprises a reduced set of validated microfluidic elements. These elements have to be able to perform the basic fluidic *unit operations* required within a given application area. Such basic fluidic *unit operations* are, for example, fluid transport, fluid metering, fluid mixing, valving, and separation or concentration of molecules or particles (see Table 1). The collection of fluidic *unit operations* needed for diagnostic applications may have only little overlap with the collection needed for pharmaceutical applications[17] or for applications in micro-reaction technology.[18] In some cases detection methods will also belong to the basic set of microfluidic operations, and in other cases not. Nevertheless, in all cases the user of a platform has to be able to readily combine the elements within a given platform in order to implement an assay for diagnostic applications or to screen for new compounds in pharmaceutical applications.

More important than providing a totally complete set of fluidic *unit operations* within a platform is the fact that all elements have to be amenable to a well established fabrication technology. Furthermore, all elements of a platform have to be connectible, ideally in a monolithically integrated way or at least by a well defined, ready-to-use interconnection and packaging process. If a platform allows a seamless and simple integration of different fluidic elements in a monolithic way, *e.g.*, without sophisticated additional packaging techniques, this provides a significant advantage compared with other platforms. Thus thinking about microfluidic platforms involves also at least one validated fabrication technology to create complete systems out of the elements. This results in a definition of a microfluidic platform as follows.

Table 1 Common features of microfluidic platforms

Microfluidic *unit operations*	Fabrication technology
• Fluid transport • Fluid metering • Fluid valving • Fluid mixing	Validated manufacturing technology for the whole set of fluidic *unit operations* (prototyping and mass fabrication)
• Separation • Concentration/ amplification/ accumulation	Seamless integration of different elements • Preferable in a monolithic way
• Detection/readout	• Or by a well defined easy packaging technique
• Reagent storage • Incubation • …	

A microfluidic platform provides a set of fluidic *unit operations*, which are designed for easy combination within a well defined (and low cost) fabrication technology. The platform allows the implementation of different application specific systems (assays) in an easy and flexible way, based on the same fabrication technology.

This paper is intended to give an overview of microfluidic platforms that have been developed up to now. We will thereby focus only on platforms for lab-on-a-chip applications, being aware that there are also other possible fields of applications for microfluidic platforms like micro-process engineering or micro-dosage systems. However, also in the field of lab-on-a-chip systems we cannot cover all the microfluidic platforms which are known from literature. It is, furthermore, not intended to assess the different platforms by their value to the industry or to the research community. We rather want to stress the microfluidic platform concept by use of some examples that are the most sophisticated today and thus clarify the strength of the approach.

I Capillary driven test strips

Test strips or "lateral flow assays", as they are also called, have been well known in the diagnostic field since the 1960s, representing the "state-of-the-art" with billions of units being produced at the lowest costs. Although this can be regarded as the most successful microfluidic platform for lab-on-a-chip applications in terms of the number of commercialized products (*e.g.*, diabetes testing, pregnancy testing, *etc.*), hardly any publication exists from a microfluidic point of view, and this despite the fact that the complexity of test strips varies from a single fleece (*i.e.* non-woven material featuring high capillarity) for, for example, pH measurement to very complex and partially also microstructured configurations of multiple fleeces that enable the implementation of more complex tests like immunoassays.

Unit operations on the platform

The basic principle of the platform is passive *liquid transport via* capillary forces within the capillaries of a fleece or a microstructured layer. The liquid samples are loaded into a start reservoir from where they penetrate the underlying fleeces. Another method, especially applied in patient self-testing applications, is the direct capillary filling of the strip from the sampling point. For blood diagnostic assays, for example, the test strip is directly contacted with the blood spilled out of the finger tip that has been previously pricked with a lancet. Within these test strips, the whole blood sample is first *filtered* in a separation fleece, holding back the blood cells, [19] as depicted in the exemplary immunoassay test strip in Fig. 1.

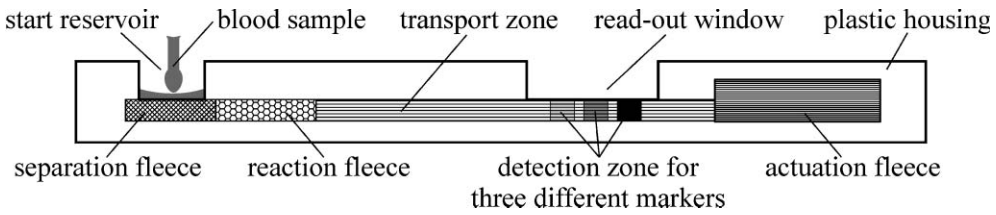

Fig. 1 Simplified cross section of a typical capillary driven immunoassay test strip.

The separation fleece is placed directly underneath the start reservoir into which the blood sample is applied.

Typically, *reagent storage* is carried out in terms of dried reagents that have been pre-deposited into the fleeces during fabrication. *Dissolving* these reagents is done by *incubating* the liquid in a reaction fleece. Therefore, different zones within the test strip, exhibiting different wetting properties, are required. The dry reagent is placed in a micro-chamber featuring, for example, a pillar structure and a low contact angle for fast priming. The propagation of the liquid meniscus is slowed down as soon as it reaches the subsequent "time gate" with an increased contact angle and, consequently, a reduced capillary force. The time for the dissolution of the dry reagent is set by the length of the time gate and ends as soon as the liquid reaches the next zone featuring a decreased contact angle, speeding up the flow again.

Metering of liquids is an important unit operation for quantitative assays. Within a test strip, metering is achieved by the defined volumes of the fleeces and microstructures. The liquid flow stops automatically, as soon as the actuation fleece (Fig. 1) is fully wetted with liquid. This way the amount of liquid that has passed the detection zone is well defined. In order to have an optimum sensitivity, however, a maximum volume of labelled sample should pass the detection zone. Therefore, the capillarity of the input zone (separation and labelling fleece) should be lower than the capillarity of the actuation fleece, ensuring a complete drainage of the sample into the actuation fleece before the liquid propulsion terminates. The only thing that has to be ensured is that the start reservoir is initially filled with enough sample liquid, *i.e.*, the volume of the complete test strip, to ensure the proper function of the strip.

The results from a test strip assay are mostly read out by optical markers. Since the concentration of those markers within the sample liquid is potentially small, they have to be *accumulated* within the detection zone. The sample volume passes the detection zone with an adequate flow rate, ensuring the non-diffusion limited binding of the marked sample molecules to the immobilized capture molecules in the detection zone. A remarkable signal is gained after a multiple of the detection zone volume has passed the immobilized molecules. Besides fluorescent markers, which require a test strip reader with some optical components, the *reading of assay results* with the naked eye is also possible. This is of interest for all applications, where a cheap and fast readout is required. A manual readable signal is produced by binding small gold or latex particles to the detection molecule, which accumulate at the detection zone and colour it. However, only clear and binary signal generating assays, such as pregnancy tests, are capable using the manual readout. Some assays are also read out using electrochemical mechanisms. The glucose concentration of a blood sample is determined by measuring the electrical charge generated during the enzymatic oxidation of glucose to gluconic acid, for example. The test strip reader applies an external electric potential and measures the current which is a function of the generated numbers of electrons.

Application examples

A huge number of assays have been developed on the capillary test strip platform during the past 40 years and are mainly published in clinical diagnostics and immunological journals. Here, the reader will only be encouraged not to lose sight of this gold-standard microfluidic platform in terms of costs and already implemented lab-on-a-chip applications.

Several applications based on the test strip platform, especially for developing countries, have been shown recently.[20] Especially, purely disposable test carriers, which do not need any electricity for carrying out the test and can be read out visually, are destined for this field of application. Rapid immunochromatographic strip (ICS) tests for sexually transmitted infections like gonorrhea and syphilis have been successfully implemented on the test strip platform. Also, test strips for the detection of *Legionella* bacteria from environmental cooling tower samples, substituting the need for running an agarose gel after the standard PCR (polymerase chain reaction), have been shown.[21] The multiplex-nested PCR is performed within a standard thermal cycler and the results are subsequently read out in a lateral flow assay *via* colloidal gold labelling and visual inspection. This makes the complex and error-prone readout *via* running an agarose gel obsolete.

Strengths and challenges of the platform

The possibility of performing an automated on-site measurement using a cheap and small disposable test strip, combined with the simple actuation principle that does not need any energy supply, gives the platform a huge potential for point-of-care and patient self-testing applications. Besides simple binary tests, also more complex immunoassay protocols have been implemented recently. Thus, the test strip platform is setting a benchmark in terms of costs and integrated, automated assay implementation for all microfluidic platforms discussed within this paper.

Drawbacks of the platform certainly arise from its simplicity. Assay protocols within capillary driven systems follow a fixed process scheme, imprinted in the microfluidic channel design. Passive liquid propulsion by capillary forces only cannot be influenced actively once the process is started.

As a consequence, the exact timing of the assay steps depends on variations in viscosity and surface tension of the sample. Other crucial unit operations are metering and incubation, the accuracy of which is limited, and mixing, which cannot be accelerated on the test strip platform. Therefore the precision of the assay result for example is in the order of 10%, which is not always sufficient for future challenges in the implementation of more complex diagnostic assays.

A further critical point is the long term stability of the wetting properties inside the fleeces or microstructures. Usually, the materials are plasma treated or coated by an additional layer to ensure the desired contact angle and thus wetting behaviours. These coatings or surface activations have to be stable at different temperatures and over a long period of time as they define the test strip life time.

II Microfluidic large scale integration (LSI)

Many pressure-driven microfluidic components and systems have been presented within the past and are commercialized today.[22] Within this section, one of the most prominent and inspiring pressure driven platform concepts is discussed. The microfluidic large scale integration platform (LSI) arose together with a novel fabrication technology for microfluidic channels, called soft lithography. Using that technology, the monolithic fabrication of all necessary fluidic components within one single elastomer material (PDMS) became possible, similar to the silicon based technology in microelectronics. PDMS (polydimethylsiloxane) is an inexpensive but still powerful material, offering several advantages compared with silicon or glass. It is a cheap, rubber-like elastomer with good optical transparency and biocompatibility. It can be structured using the soft lithography technique based on replication molding on micromachined molds. It was first used by George Whiteside's group for the fabrication of optical devices[23] and stamps for chemical patterning.[24,25] Thereafter, microfluidic devices were manufactured using the PDMS-technology.[26–30] A general and detailed up to date view of the use of PDMS for different fields of applications can be found in ref.31.

Since then, however, PDMS has been used as a merely passive material for the construction of microfluidic channels only. The strength of the technology really became obvious when Stephen Quake's group expanded the technology towards the multi-layer soft-lithography process, MSL.[32,33] With this technology, several layers of PDMS can be hermetically bonded on top of each other, resulting in a monolithic, multi-layer PDMS structure. Today, this technology is pushed forward by the company Fluidigm Corporation, USA.[34]

Unit operations on the platform

Based on the high elasticity of PDMS, the basic microfluidic unit operation is a valve which is made of a planar glass substrate and two layers of PDMS on top of each other. The lower elastomer layer contains the fluidic ducts and the upper elastomer layer features pneumatic control channels. To make a *microfluidic valve*, a pneumatic control channel crosses a fluidic duct as depicted in Fig. 2, left. A pressure p applied to the control channel squeezes the elastomer into the lower layer, where it blocks the liquid flow. Because of the small size of this valve, of the order of $100 \times 100\ \mu m^2$, a single integrated fluidic circuit can accommodate thousands of valves. Compared with the development in microelectronics, this approach is called microfluidic large scale integration, LSI.[35]

The valve technology called NanoFlex™ is the core technology of the complete platform. Placing two of such valves at the two arms of a T-shaped channel, for example, creates a *fluidic switch* for the routing of liquid flows between several adjacent channels. *Liquid transport* within the fluid channels can be accomplished by use of external pumps, while the PDMS multi-layer device works merely passively, controlling the externally driven liquid flows with the integrated valves. Also, an integrated pumping mechanism can be achieved by combining several micro-valves and actuating them in a peristaltic sequence (Fig. 2(b)).

Metering of liquid volumes can be achieved by crossed fluid channels and a set of microvalves. Addressed by a multiplexer, the liquid is loaded into a certain fluid channel and segmented into separated liquid compartments by pressurizing the control channel.

Also, *mixing* can be achieved using the above described pumping mechanism (Fig. 2(c)) by the subsequent injection of the liquids into the fluidic loop through the left inlet (right outlet valve is closed). Afterwards, the inlet and outlet valve are closed and the three control channels on the orbit of the mixing loop are displaced with a peristaltic actuation scheme, leading to a circulation of the mixture within the loop.[33] Thereby the liquids are mixed and afterwards flushed out of the mixer by a washing liquid. By using this mixing scheme, increase of the reaction kinetics of surface binding assays by nearly two orders of magnitude has been demonstrated.[36]

The key feature to tap the full potential of the large scale integration approach is the multiplexing technology, allowing

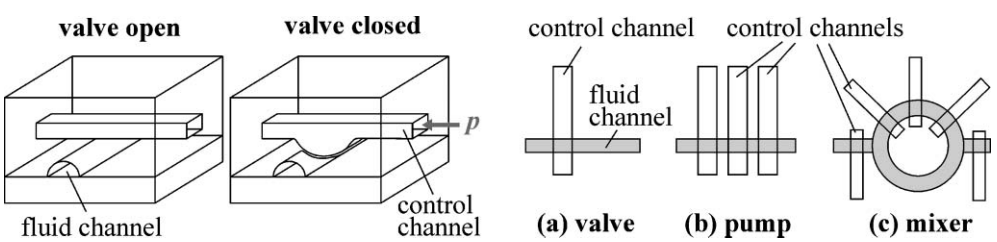

Fig. 2 Construction of the main unit operations on the multi-layer PDMS based LSI platform. The NanoFlex™ valve as depicted on the left can be closed by applying a pressure p to the control channel. Therewith, microfluidic valves (a), peristaltic pumps (b) and mixing structures (c) can be designed.

the control of N fluid channels with $2\log_2 N$ control channels only. Based on this principle, a microfluidic storage device with 1000 independent compartments of approximately 250 pL volume and 3574 microvalves has been demonstrated.[35]

Application examples

Protein crystallization based on the free interface diffusion method (FID) is a promising application on the LSI platform.[37] The method is based on the counter-diffusion of two liquid phases, namely the protein solution and the precipitant solution, at their contact interface (see Fig. 3). During the diffusion process, the concentration profile changes and crystal growth is initiated as soon as the appropriate conditions are met. Within the small dimensions of the microfluidic crystallization structure, a stable interface between the two liquids can be accomplished, ensuring diffusion based mixing between the two phases only. The crystallization experiments are performed in parallel within 48 unit cells on the microfluidic LIS chip, facilitating 144 different simultaneous crystallization reactions while consuming 3.0 μL of protein solution only.[38,39] The protein crystallization technology on the LSI platform has been commercialized by the company Fluidigm (Topaz® technology).

A second application example on the microfluidic LSI platform is the extraction of nucleic acids from a small number of cells.[40,41] For the extraction of DNA from a cell suspension, the cell membrane has to be destroyed first (lysis of the cell). Afterwards, the DNA is specifically separated from the residual cell constituents within the solution. This extraction protocol is completely implemented on the microfluidic platform using the basic unit operations for valving, metering, mixing and switching of fluids. Purified genomic DNA from less than 28 bacterial cells (*E. coli* culture) could be successfully isolated on the platform. This corresponds to an increase in sensitivity of this process three to four orders of magnitude over that of conventional methods.[40] Based on that technology, a nucleic acid processor for complete single-cell analysis is under way.[42–44]

Strengths and challenges of the platform

The microfluidic LSI platform certainly has the potential to become one of the foremost microfluidic platforms for highly integrated applications. It is a flexible and configurable technology which stands out owing to its suitability for large scale integration. The PDMS fabrication technology is comparably cheap and robust and it can be used to fabricate disposables. Reconfigured layouts can be assembled from a small set of validated unit operations and design iteration periods for new chips are of the order of days. Some of the system functions are hardware defined by the fluidic circuitry but others, like process sequences, can easily be programmed from outside.

Limitations of the platform are related to the material properties of PDMS: for example, chemicals which are not inert to the elastomer cannot be processed, or elevated temperatures such as in micro-reaction technology are not feasible. Also, the implementation of applications in the field of point-of-care diagnostics, where often a hand-held device is required, seems not to be beneficial using the LSI platform. The external pressure sources and valves have to be shrunk to a smaller footprint, which is technically feasible, of course, but the costs would be higher in comparison with other platform concepts.

III Centrifugal microfluidics

The approach of using centrifugal forces to process samples and reagents dates back to the end of the 1960s.[45,46] At that time, centrifugal analyzers had first been used to transfer and mix a series of samples and reagents in the volume range from 1 μL up to 110 μL into several cuvettes followed by spectrometric monitoring of reactions and real-time data processing. At the beginning of the 1990s, the company Abaxis[47] developed the portable clinical chemistry analyzer.[48,49] The system consists of a plastic disposable rotor for processing the specimen, dried reagents pre-loaded to the cartridge and an analyzer instrument for actuation and readout.

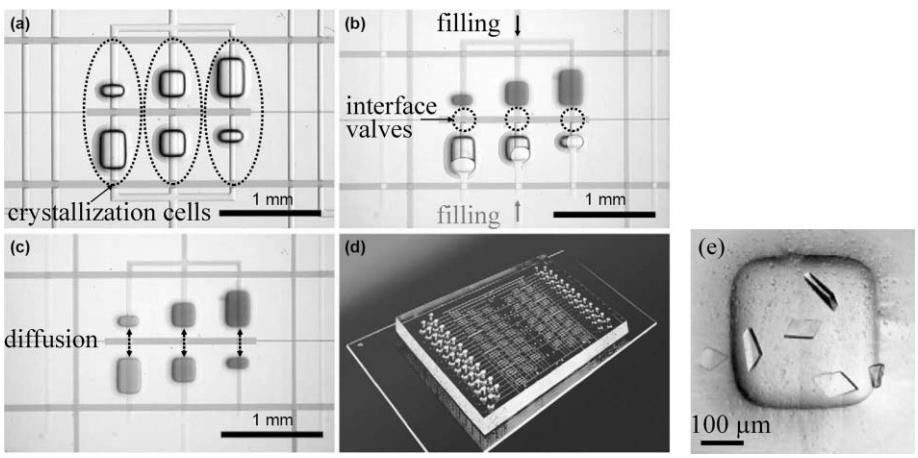

Fig. 3 Microfluidic realization of a free interface diffusion (FID) protein crystallization assay, based on the large scale integration platform (LSI).[39] One unit cell consists of three crystallization cells for crystallization with different mixing ratios (a). They are initially filled with liquid while the central interface valve is closed (b). Afterwards, the interface valve is opened to allow diffusive mixing between the coupled chambers (c). The chip (d) consists of 48 cells for protein crystallization. An example for a protein crystal grown in the LSI chip is depicted on the right (e). (Reprinted with permission from ref. 39.)

A new generation of centrifugal devices emerged from the technical capabilities offered by microfabrication and microfluidic technologies.[50–53] Length scales of the fluidic structures in the range of a few hundred micrometres allow parallel processing of up to one hundred units assembled on a disk. This enables a high throughput of many tests by highly parallel and automated liquid handling. In addition, the new opportunities arising from the miniaturization of the centrifugal fluidics cut down assay volumes to less than 1 μL. In particular, fields such as drug screening, where precious samples are analyzed, benefit from the low assay volumes. A review of the theory of and applications on the centrifugal microfluidic platform has been published recently.[54]

Unit operations on the platform

Liquid transport is initiated by the radial outwards directed centrifugal force f_ω, which can be scaled over a wide range by the frequency of rotation ω together with the flow resistance of the fluidic channels. Small flow rates of the order of nL s^{-1}, as well as high throughput continuous flows up to 1 mL s^{-1},[55] can be generated. So, scaling of flow rates over 6 orders of magnitude and independent of the chemical composition, ionic strength, conductivity or pH value of the liquid can be accomplished, opening a wide field of possible applications.

Liquid *valves* can basically be constructed by three different microfluidic structures on the centrifugal platform, as depicted in Fig. 4. A very simple valve arises at the sudden expansion of a microfluidic channel, for example into a bigger reservoir (A). The valving mechanism of this capillary valve is based on the energy barrier for the breaking of the meniscus, which is pinned at the sharp corner. This barrier can be overcome under rotation due to the centrifugal pressure load of the overlying liquid plug.[51] For a given liquid plug position and length, *i.e.*, for a given set of geometric parameters, the valve can be influenced by the frequency of rotation only and a critical burst frequency ω_c can be attributed to every valve structure. Another possibility to stop the liquid flow within a channel is local hydrophobic coating of the channel walls (B). Also this valve is opened as soon as the rotational frequency exceeds the critical burst frequency ω_c. A third method is based on a hydrophilic U-shaped siphon channel, wherein the two liquid–gas interfaces are levered at high frequencies of rotation (C). Below a critical frequency ω_c, however, the right meniscus proceeds beyond the bend, thus generating a net radial length allowing the centrifugal force to drain the complete liquid from the siphon.

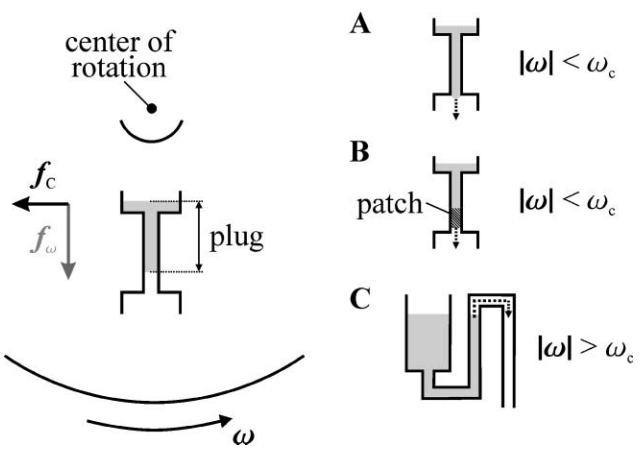

Fig. 4 Principal centrifugal approach and schematic sketch of the three valving techniques on the centrifugal platform. (A) geometric capillary valve, (B) hydrophobic valve and (C) hydrophilic siphon valve.

An alternative approach to the control of liquid flows on the centrifugal platform is followed by the company Spin-X technologies, Switzerland.[56] A laser beam individually opens fluidic interconnects between different channel layers on a plastic substrate (Virtual Laser Valve, VLV). This enables an online control of the liquid handling process on the rotating module for adjusting metered volumes and incubation times within a wide range. For this reason, the Spin-X platform works with a standardized fluidic cartridge that is not custom made for each specific application, but can be programmed just before the measurement, or even online during a running process.

Combining one of the above mentioned valve principles at the outer end of a chamber and an overflow channel at the inner end results in a *metering* structure.[57] The metered liquid portion is directly set by the volume capacity of the chamber. Owing to the high precision of micro-fabrication technologies, small coefficients of variation (standard deviation/mean value), *e.g.*, a CV $< 5\%$ for a volume of 300 nL[58] and also metered volumes of only 5 nanolitres have been achieved.[59] By arranging several metering structures interconnected *via* an appropriate distribution channel, simple aliquotting structures can be realized.[60] These structures split a sample into several defined volumes, enabling the conduction of several assays (with different volumes) from the same sample in parallel.

Different *mixing* schemes have been proposed on the centrifugal platform. Considering mixing of continuous liquid flows within a radially directed rotating channel, the perpendicularly directed Coriolis force f_C automatically generates a transverse liquid flow.[55,61,62] A continuous centrifugal micromixer, utilizing the Coriolis stirring effect, showed an increasing mixing quality towards very high volume throughputs of up to 1 mL s^{-1} per channel.[55] Besides the mixing of continuous liquid flows, the homogenization of discrete and small liquid volumes located in chambers is also of importance, especially when analyzing small sample volumes (batch mode mixing). One possibility to enhance the mixing is the active agitation of the liquid within a mixing chamber by inertia related shear forces, induced by a fast change of the sense of rotation (shake-mode-mixing).[57] This method leads to reduced mixing times of the order of several seconds, compared with several minutes for pure diffusion based mixing. A further downscaling of mixing times below one second using magnetic microparticles located in the mixing chamber has also been demonstrated.[63]

For routing (*switching*) of liquids, a switch availing itself of the transverse Coriolis force to guide liquid flows between two outlets at the branching of an inverse Y-shaped channel has been presented.[64] Depending on the sense of rotation, the Coriolis force is either directed to the left or to the right, guiding the liquid stream into one of two downstream reservoirs at the branching. An improved version of Coriolis

based switching operates on individual droplets and enables switching of small flow rates of 160 nL s^{-1} only at low frequencies down to a few Hz.[65] Another method for liquid-routing, based on different wetting properties of the continuative channels, has been reported by Gyros AB, Sweden.[66] The liquid stream is initially guided towards a radial channel, exhibiting a hydrophobic patch at the beginning. Therefore, the liquid is deflected into another, not hydrophobic, channel next to the radial one. For high frequencies of rotation, the approaching liquid possesses enough energy to overcome the hydrophobic patch and is therefore routed into the radial channel.[67]

The extraction of plasma from a blood sample (*separation*) is the prevalent first step within a complete analytical protocol starting from an untreated whole blood sample. Since blood plasma is less dense than the white and red blood cells it can be found in the upper phase, the so called supernatant, after sedimentation in the artificial gravity field under rotation. The spatial extraction of the gained plasma from the cellular pellet can be done *via* a capillary extraction channel that branches from the sedimentation chamber at a radial position where only plasma is expected.[49] Another method uses a pre-separation of the cellular and plasma phase during the sample flow through an azimuthal aligned channel of just 300 μm radial width.[68] The obtained plasma fraction is thereafter split from the cellular components by a decanting process. This centrifugal flow separation technique extracts 2 μL of plasma from a 5 μL raw blood sample within only 20 s.

Application examples

Madou *et al.* from the University of California, Irvine, showed a series of capillary valves to perform enzyme-linked immunosorbent assays (ELISAs) on the centrifugal platform.[69] The different assay liquids are contained in reservoirs connected *via* valves of different burst frequency to the reaction chamber. The capillary valves are opened subsequently by increasing the frequency of rotation. It could be shown that the centrifugally conducted assay has the same performance in terms of detection range as the conventional method on the 96-well plate, while having advantages over the conventional method such as less reagent consumption and shorter assay time.

Gyros AB, Sweden[66] use a flow-through sandwich immunoassay at nanolitre scale to quantify proteins within the Gyrolab™ Workstation. Therefore, a column of pre-packed and streptavidin-coated micro-particles is integrated in each of, in total, 112 identical assay units on the microfluidic disk. Each unit has an individual sample inlet and a volume definition chamber that leads to an overflow channel. Defined volumes (200 nL) of samples and reagents can be applied to the pre-packed particle column. The laser induced fluorescent (LIF) detector is incorporated into the Gyrolab™ Workstation. Using that technology, multiple immunoassays have been carried out to determine the imprecision of the assay result. The day-to-day (total) imprecisions (CV) of the immunoassays on the microfluidic disk are below 20%.[70] The assays were carried out within 50 min while, in comparison, the traditional ELISA in a 96-well plate typically takes a few hours, with sample volumes of a few hundred microlitres.

A fully integrated colorimetric assay for the determination of the alcohol concentration in human whole blood has been shown on the centrifugal Bio-Disk platform.[58] After loading the reagents into the reagents reservoir, a droplet of untreated human blood derived from a finger tip is loaded into the inlet port of the microstructure. To enforce rapid mixing within the reaction chamber, the sense of rotation is frequently reversed for 10 s, leading to a homogeneous mixture (shake-mode mixing). Thereby an enzymatic reaction is initiated, changing the color of the mixture depending on the alcohol concentration. After sedimentation of the residual blood cells, the absorbance is monitored in real-time *via* a laser beam that is reflected into the disk plane on integrated V-grooves.[71] By using this automated assay and readout protocol the concentration of alcohol in human whole blood could be determined within 150 s only. The results were comparable to

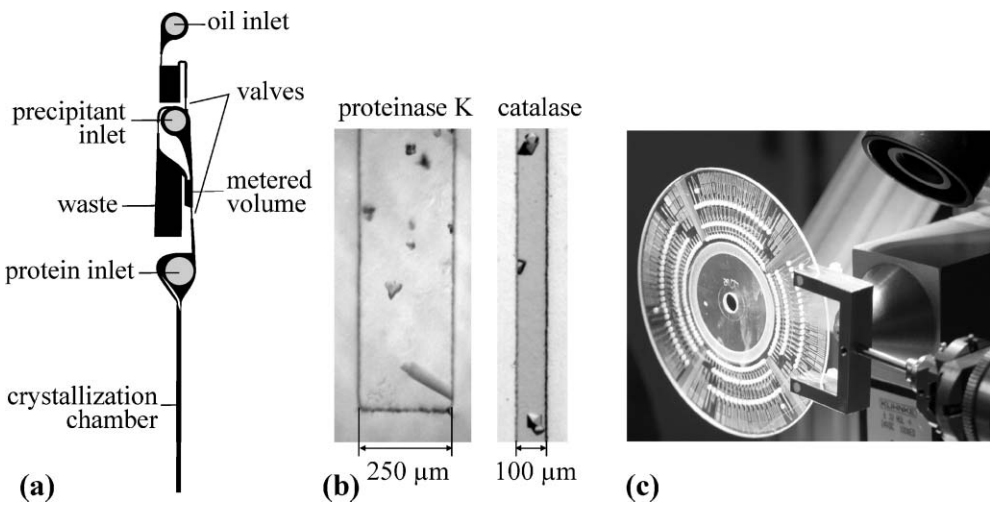

Fig. 5 Microfluidic realization of a free interface diffusion (FID) protein crystallization assay, based on the centrifugal microfluidic platform (a).[59] The three liquids (protein, metered precipitant, oil) are subsequently transported into the crystallization chamber triggered by hydrophobic valves. Two examples of protein crystals are depicted in the middle (b). The complete microfluidic disk (c) enables up to 100 crystallization experiments in parallel (picture shows readout in the X-ray beamline).

common point-of-care tests and required a minute blood volume of just 500 nL.

Also a protein crystallization assay has been demonstrated on the centrifugal microfluidic platform, as depicted in Fig. 5.[59] First, a defined volume of the protein solution is dispensed into the protein inlet and transported into the crystallization chamber. Afterwards, the pre-loaded precipitant is metered under rotation and transferred into the crystallization chamber as soon as the hydrophobic valve breaks. In the last step, the pre-loaded oil is released at a still higher frequency and placed on top of the liquid stack within the crystallization chamber, to prevent evaporation. The successful crystallization of proteinase K and catalase could be demonstrated.

Strengths and challenges of the platform

The modular setup of the system with cheap, disposable and easy exchangeable plastic cartridges is certainly one major advantage of the centrifugal microfluidic platform. The cost efficient fabrication predominantly originates in the simple and passive microfluidic elements that can easily be combined in a monolithic way within the same fabrication process. Those elements allow the implementation of all needed unit operations to perform complex assay protocols in an automated way. Owing to the rotational symmetry of the disks, optionally a high degree of parallelization can be achieved. All processes are controlled by the frequency of rotation of one single macroscopic rotary engine. In addition, the centrifugal microfluidic platform can be easily applied to a wide range of different applications due to the fact that it allows scaling of the pulse-free flow rates by 6 orders of magnitude.

As soon as any additional actuation or sensing function is required on the module while it is rotating, things become tricky from a technical point of view if a contact free interface is not applicable. The platform also lacks flexibility compared with others that allow on-line programming of fluidic networks within one piece of hardware that fits all. Most of the logic functions, as well as their critical frequencies, are permanently imprinted into the channel network.

IV Electrokinetic platforms

Electrokinetic pumping and particle manipulation principles are based on surface forces and thus gain impact within the micro-dimensions due to the increased surface to volume ratio. This advantage combined with the simple setup of electrokinetic systems, which basically consist of microfluidic channels and electrodes, explains the early advent of microfluidic lab-on-a-chip applications based on the electrokinetic platform. They focused on the analysis of chemical compounds *via* electrophoretic separation within microchannels (capillary electrophoresis, CE).[72–75]

Unit operations on the platform

Fluid propulsion (*fluid transport*) on the electrokinetic platform is based on the movement of the liquid layer right at the interface to the solid phase (electric double layer) initiated by an external voltage (Fig. 6A). Standard channel materials for electrokinetic actuation are silicon or glass, which possess negatively charged surfaces thus causing a surplus of positively charged liquid molecules in the double layer close to the channel walls. As soon as an electric potential is applied along the channel, the positively charged liquid molecules are attracted by electrostatic forces and thus move towards the negative electrode. As a result of the viscous coupling the bulk liquid is dragged by the moving layer and a planar velocity profile (v) evolves. This is a major difference compared with the parabolic velocity profile of pressure driven flows. Consequently, a rectangular shaped liquid plug with limited dimensions along a micro-channel gets increasingly widened within a pressure driven flow, while it keeps its original flat shape within an electroosmotic flow. So sample dispersion is drastically reduced in electroosmotic flows, making it the method of choice for chromatographic analysis.

Based on the electroosmotic flow, *metering* of volumes down to the picolitre range at the intersection point of a vertical and a horizontal channel can be achieved. Both channels are filled with a system buffer at the beginning. Afterwards, a sample liquid is injected and crosses the intersection point by applying a voltage to the vertical channel. Subsequently, the flow is stopped and an electric field is applied to the horizontal channel, causing a liquid flow of the buffer which displaces the small plug at the junction and thus meters a volume corresponding to the dimensions of the intersection area. This method is used within capillary electrophoresis tests for the injection of the sample into the separation channel.

The *mixing* of two co-flowing streams has been shown on the electrokinetic platform by applying an ac voltage to a pair of coplanar meandering electrodes configured parallel to the channel.[76] A mixing time of 0.18 s, which is 20-fold faster than diffusion, has been reported. Also, complete process schemes comprising cell lysis, mixing and DNA *amplification* based on fluid propulsion by electroosmotic flow have been presented.[77]

Besides electroosmosis, other effects also occur within the electric field as soon as the liquid contains electrically charged particles or molecules (Fig. 6B). They are attracted (F) by one of the electrodes, depending on their charge and valence, and consequently move towards the electrode also in a stationary

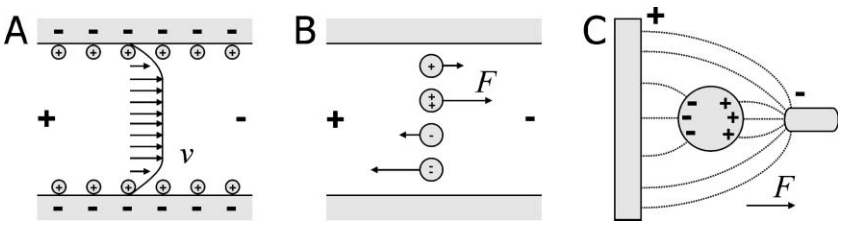

Fig. 6 Basic electrokinetic effects. A: Electroosmotic flow (EOF), B: electrophoresis (EP), C: dielectrophoresis (DEP).

surrounding liquid. The velocity of the molecule depends on its charge and size and enables the distinction between different species. This effect is called electrophoresis (EP) and is used for chromatographic *separation*.

A modification of electrophoresis is free field electrophoresis, which enables the continuous separation of a mixture according to charge with subsequent collection of the sample band of interest.[78] Therefore, the electric field is applied perpendicular to the superposed pressure driven flow within a broad and flat microchamber. While passing this extraction chamber, the species contained in the sample flow are deflected, depending on their charge, and thus exit the chamber through one of several outlets.

The third electrokinetic effect is based on the temporary charging of intrinsically uncharged particles within an alternating current (ac) electrical field. This can either be a time changing or a locally changing electrical signal, *i.e.*, non-uniform electric field, as depicted in Fig. 6C. This effect is applied in many fields of application, *e.g.*, for the controlled separation and trapping of sub-micron bioparticles,[79] for the fusion of cells[80] or the separation of metallic from semiconducting carbon nanotubes.[81]

Application examples

Capillary electrophoresis systems were the first micro total analysis systems and emerged from the analytical chemistry field at the end of the 1990s.[72–75] They arose from the idea that chemical measurements within sub-millimetre reaction vessels enable the conduction of more experiments per chip consuming less (expensive) reagents within shorter reaction times (due to the faster diffusion processes). However, a lower limit of the reaction vessel dimensions exists as well since the number of molecules within the sample would vanish when downscaling too far. In conclusion, the 10 μm to 100 μm dimensional scale appears to be an interesting domain for miniature chemical analysis systems.[82]

Liquid propulsion *via* electroosmosis in combination with the injection of a sample plug into a separation channel represents the basic set-up of capillary electrophoresis (CE) systems. The sample is afterwards separated by electrophoresis, leading to spatially separated bands of the different species within the sample (Fig. 7). Today, assays based on the electrokinetic platform that use capillary electrophoresis are commercially distributed by Caliper Life Sciences[83] and Agilent Technologies[84] for DNA and protein analysis.

Strengths and challenges of the platform

The electroosmotic actuation of liquid flows enables pulse free pumping without any moving part. Additionally, no dispersion occurs in the EOF flow and thus sample plugs are not broadened during chromatographic separation. These two advantages give reason to the successful application of capillary electrophoresis for DNA and protein quantification. Miniaturization of electrophoretic analysis enables the automation and parallelization of tests with small dead volumes, thus reducing the required amount of sample. Furthermore, higher voltages can be used for the separation and the dissipation of heat is increased compared with macroscopic systems due to the higher surface-to-volume ratio. Overall, miniaturized electrophoresis enables the fast and efficient analysis of biomolecules.

Drawbacks of the technology can be seen in the need for high performance detection technologies due to the reduced volumes and thus signals. Also, technical problems arise in capillary electrophoresis systems due to pH-gradients arising over time or during operation, streaming currents which counteract the external electric field and gas bubbles that can occur due to electrolysis at the electrodes. In addition, high voltages are needed that can hardly be generated in, for example, mobile hand-held devices.

V Droplet based microfluidic platforms

The principal idea behind droplet based or digital microfluidic systems is the use of single droplets as reaction confinements for biological assays or chemical reactions. Dominant interfacial and surface tensional forces in the micro-dimension enable the precise generation and spatial stabilization of these droplets. Since the droplets are kept isolated within an immiscible surrounding fluid like air or oil, lateral dispersion (Taylor dispersion) can be avoided while moving the droplets to different locations. A multitude of parallel screening

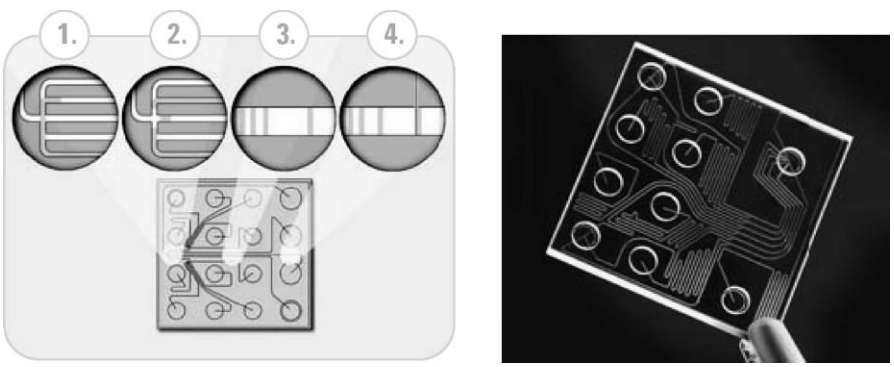

Fig. 7 Microfluidic realization of capillary electrophoresis analysis on the electrokinetic platform.[84] After the sample has been transported to the junction area (1) it is metered by the activated horizontal flow and injected into the separation channel (2). Therein, the sample components are electrophoretically separated (3) and read out by their fluorescence signal (4). The complete microfluidic CE-chip is depicted on the right. (© Agilent Technologies, Inc. 2007. Reproduced with Permission, Courtesy of Agilent Technologies, Inc.)

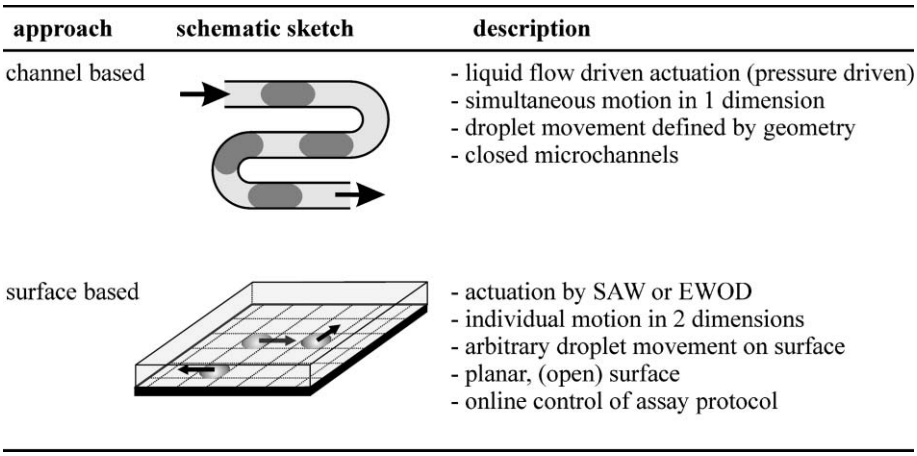

Fig. 8 Description of the two basic setups for droplet based microfluidic platforms.

reactions, each consuming only a minute amount of reagent, is enabled inside several small sized droplets on the platform.

Droplet based microfluidic systems can be fundamentally divided into two basic setups, the channel based and the planar surface approach as described in Fig. 8. The channel based systems are mostly pressure driven with the droplet generation and manipulation relying on actuation *via* liquid flows within closed microchannels. On the planar surface based platforms, droplets can be arbitrarily moved in two dimensions representing planar programmable laboratories on-chip. They are actuated by electrowetting (EWOD) or surface acoustic waves (SAW) respectively.

Pressure driven unit operations and applications

The pressure driven, droplet based platform relies on a two-phase fluid flow through the microchannels. The two immiscible phases are dispersed into each other so that a sample fluid (*e.g.* aqueous solution) forms plugs of a certain length, separated by the carrier fluid (*e.g.* oil) along the channel. This flow scheme is called segmented flow, since the size of the inner phase droplet exceeds the cross sectional dimensions of the channel leading to squeezed fluid plugs. The two phase flow is pumped throughout the channels by an externally applied pressure.

The most elementary unit operation on the pressure driven, droplet based platform is the initial generation of the droplets. This step can also be considered as *metering*, since the liquid volumes involved in the latter reaction within the droplet are defined during the droplet formation process. Generally, two different microfluidic structures have been reported for a controlled droplet generation, the flow focusing structure[85,86] and the T-shaped junction,[87] respectively. The formation of double emulsions, *e.g.*, water-in-oil-in-water (W/O/W), has been shown in a serial arrangement of T-junctions[88] or within more complex interleaved microcapillaries arrangements[89] also. The size of the droplet is influenced by the channel dimensions and the strength of the shear forces at the channel junction (higher shear forces lead to smaller droplets) for both droplet formation mechanisms.

To use droplets inside channels as reaction confinements, the different liquid streams have to be loaded into the droplets first. A method to combine three different sample liquid streams by a sheath flow arrangement with subsequent injection as a common droplet into the carrier fluid has been shown by the group of Rustem F. Ismagilov at the University of Chicago, USA.[90] Different concentrations and ratios of two reagent sub-streams plus a dilution buffer merge into one droplet and perform a so called on-chip dilution.[91] The mixing ratios can be adjusted by the volume flow ratio of the three streams (see also Fig. 9, a).

Using a combination of two opposing T-junctions connected to the same channel, the formation of droplets of alternating composition has been demonstrated.[92] This method can be used for protein crystallization or indexing within the continuous flow screening experiments on the droplet based platform, for example. By use of a similar technique, the injection of an additional reactant into a liquid plug moving through the channel at an additional downstream T-junction has also been demonstrated.[93] Not only liquid chemical reagents but also other components like cells have been loaded into droplets as reported in ref. 94. Therefore, a flow focusing device has been used with an aqueous cell suspension as the sample phase flowing through the central channel.

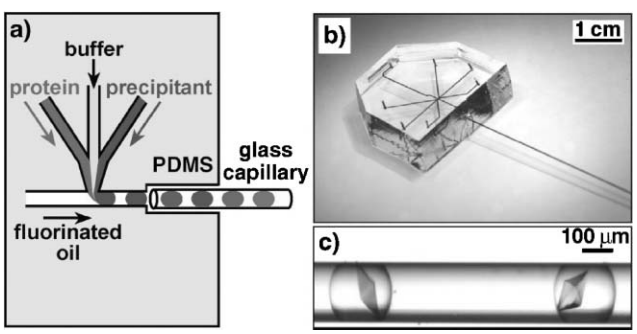

Fig. 9 Microfluidic realization of a protein crystallization assay, based on the pressure driven droplet-based platform.[109] The protein and precipitation solution are continuously injected into one droplet of adjustable volume (a) and afterwards transported into a glass capillary for crystallization (b). Re-circulating flows inside the droplets enhance mixing and induce the crystallization process (c). (Reprinted with permission from ref. 109.)

The *merging* of different sized droplets possessing different velocities to form single droplets has been demonstrated successfully.[90] In the same work, the controlled *splitting* of droplets at a channel branching point has been shown. Using a similar method, the formation of droplet emulsions with controlled volume fractions and drop sizes has been realized.[95]

Mixing inside the droplets can be accelerated by a re-circulating flow due to shear forces induced by the motion along the stationary channel wall.[96] This effect is even more pronounced if two liquids of differing viscosities are mixed within the droplet, as reported in ref. 97. Based on the recirculation flow, a mixing scheme for the pressure driven, droplet based platform has been proposed using wound microchannels.[98] Within each channel curvature the orientation between the phase pattern in the droplet and the direction of motion is changed so that the inner recirculation leads to a stretching and folding of the phases. Under favourable conditions, sub-millisecond mixing can be achieved and has been employed for the multi-step synthesis of nanoparticles, for example.[93] A detailed and theoretical description of this mixing effect is given in ref. 99.

Besides the mixing within liquid droplets dispersed into another liquid carrier phase, also mixing within the carrier phase can be accelerated by a segmented flow. The injection of gas bubbles into a continuous liquid stream forming a segmented gas–liquid flow has been described by Klavs Jensen and his group at the MIT.[100,101] The gas bubbles are introduced into the liquid flow and initiate recirculation flows within the liquid segments in between due to the motion along the channel wall. The gas bubbles can be completely separated from the liquid stream using a planar capillary separator after the reaction is finished. Using that technology, the synthesis of colloidal silica particles has been demonstrated.[102] Another microfluidic mixing scheme based on a gas–liquid segmented flow uses an additional repeated separation and re-combining of the channel.[103]

The *incubation* time of the reagents combined inside a droplet at the injection position can easily be calculated at a certain point of observation from the travelling distance of the droplet divided by the droplet velocity. Thus, the incubation time can be monitored, time resolved, by simply scanning along the channel from the injection point to more downstream positions. This is a unique feature of the platform and enables the investigation of the kinetics of chemical reactions of the order of some milliseconds only.[91] On the other hand, incubation times of the order of a week for storing applications have been demonstrated.[104] This is enabled by the droplet compartments that are separated by the carrier fluid which prevents evaporation and diffusion. Using this approach, several 60 nL liquid droplets containing one or a few cells were generated within a microfluidic chip and afterwards flushed into a Teflon capillary tube for cultivation. The cell densities were still as high as in conventional systems after 144 hours of growth within the droplets.

Additional unit operations based on charged droplets and electric fields have been added to the droplet based pressure driven platform by David A. Weitz and co-workers.[105] Using dielectrophoresis, the sorting of single drops out of a droplet train (*switching*) at rates up to 4 kHz has been shown, for example.[106] The pressure driven droplet based technology enriched with electric field based unit operations is currently commercialized by the company Raindance Technologies, USA.[107]

Protein crystallization has been implemented on the pressure driven, droplet based platform by Ismagilov and co-workers.[108,109] Droplets from three liquids, namely the protein solution, a buffer and the precipitant, are dispersed into the oil carrier phase (Fig. 9). The precipitant concentration inside the droplet is adjusted *via* the buffer and precipitant flow rate, respectively. Therewith, different concentrations are generated and transferred into a glass capillary for later X-ray analysis.[110] Non-specific protein adsorption to the liquid–liquid interface can be suppressed by adding certain surfactants to the carrier phase.[111] The effect of mixing on the nucleation of protein crystallization has been investigated by combining the described crystallization structure with a winding mixing channel.[112] Fast chaotic mixing has been found to be favorable for the formation of well-formed proteins within the droplets.[113] Besides the described method for crystallization, also an alternative process in which the concentration within one droplet is changed over time has been presented.[109,114] In this case, a carrier liquid which enables diffusion between the droplets, *i.e.*, a water permeable liquid, is selected. Alternating droplets of protein and precipitant on the one hand and a high concentration salt solution on the other hand are generated using two opposing droplet generation structures.[92] Water diffuses through the oil carrier phase from the low salt concentration, *i.e.*, protein containing droplet, to the high concentration droplet. This steadily increases the concentration within the protein containing droplet until suitable crystallization conditions are achieved.

Recent developments on the pressure driven, droplet based platform aim at high throughput screening applications. Therefore a large number of droplets, each containing a different reagent, are separated and surrounded by a fluorinated carrier fluid within a micro-capillary. In order to prevent coalescence during possibly long storing times, a gas bubble is injected between the droplets as an additional separation phase.[115] Based on this three phase liquid–liquid–gas system, reliable and high throughput screening assays can be performed which could be an alternative for well plates in the future.[116]

Electrowetting driven unit operations and applications

The electrowetting effect was first described by Gabriel Lippmann in 1875, while recent developments were initiated in the early 1990s by introducing the idea of using thin insulating layers to separate the conductive liquids from the metallic electrodes in order to eliminate electrolysis (taken from a recent review paper on electrowetting:[117]). That paved the way for the application of the electrowetting effect as a liquid propulsion principle for lab-on-a-chip systems.

The ''electrowetting-on-dielectric'' (EWOD)[118] technology is based on a liquid droplet which is placed between two electrodes covered with insulating dielectric layers. An applied voltage between the two electrodes changes the contact angle. So electrowetting-on-dielectric can be simply described as a

tool to control the contact angle of conductive liquids.[119] Using the EWOD setup, a microfluidic actuation method for moving droplets has been published by the group of Michael G. Pollack from the Duke University, Durham,[120] and Chang-Jin (''CJ'') Kim from the University of California, Los Angeles (UCLA),[121] in 2000. Several individual addressable control electrodes are located on the bottom of the device to control the droplet path. They are typically arranged in two-dimensional arrays. An additional hydrophobic layer (mostly Teflon®) is applied to the insulator surface to enhance the droplet movement.

The droplet, which is enclosed between the two electrode plates, features a sufficient volume to cover parts of two addressable electrodes at all times. If a voltage is applied to one of the control electrodes covered by the droplet, the contact angle at this part of the droplet is reduced, initiating a movement of the droplet along the paths given by the activated pads. Because the path of the droplets is determined by the pattern of electric fields, the EWOD driven, droplet based platform is easily programmable. This allows an operator to perform different assays, to be run by different programs on the same piece of hardware. Besides aqueous solutions, also several other liquids, like organic solvents, ionic liquids, aqueous surfactants solutions,[122] and also biological fluids, like whole blood, serum, plasma, urine, salvia, sweat and tears,[123] have been successfully *transported* on the EWOD droplet based platform.

The dispensing, *i.e.*, initial *metering* unit operation, is probably the most critical on the EWOD driven, droplet based platform. Metered droplets can be formed from an on-chip reservoir in three steps.[123] First, a liquid column is extruded from the reservoir by activating a series of electrodes adjacent to it. Secondly, once the column overlaps the electrode on which the droplet is to be formed, all the remaining electrodes are turned ''off'' to form a neck in the column. The reservoir electrode is then activated within the third and last step, to pull back the liquid and break the neck completely to form a droplet. Using this droplet metering structure, droplets of 20 nL volume could be generated with a standard variation below 2%.[123] Since the droplet dispensing is a crucial step for the performance and accuracy of all assays on the EWOD platform, additional measures for a controlled liquid metering like on-chip capacitance measurement of volume control[124] or the use of numerical methods for the design of EWOD structures[125] have been proposed.

Also, the *merging* of droplets on three linearly aligned EWOD electrodes has been presented by contacting two initially separated droplets on a single electrode.[126] Together with droplet generation from a reservoir and the droplet transport along electrode arrays, the controlled merging and *splitting* of droplets complete the four fundamental fluidic operations considered essential in building digital microfluidic systems for lab-on-a-chip applications.[127]

The most basic type of *mixing* within droplets on the EWOD platform is an oscillation between two electrodes. Before this active mixing scheme is applied, the two droplets containing the liquids to be mixed have to be merged into a single droplet. This coalesced droplet is afterwards moved along the electrodes in an oscillating fashion to induce advectional

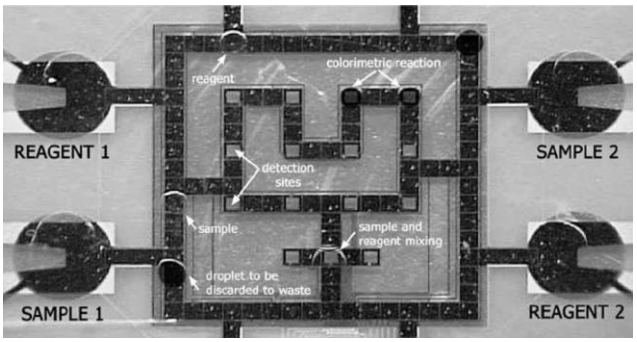

Fig. 10 Integrated lab-on-a-chip architecture for a colorimetric glucose assay, based on the droplet based electrowetting platform. Four reservoirs with injection elements are connected to an electrode circuit, where the droplets are mixed, split and transported to detection sites for readout.[123] Reproduced by permission of the Royal Society of Chemistry.

effects inside the droplet. An increasing frequency of droplet movement leads to reduced mixing times. The mixing process can be further accelerated by oscillating over a longer linear electrode array. The shortest mixing time for two 1.3 μL droplets in linear oscillation on 4 electrodes was about 4.6 s.[128] In another work, the mixing times could be further reduced to less than 3 s using two-dimensional arrays.[129]

Different *readout* schemes for biochemical assays have been applied to the EWOD driven, droplet based platform. Colorimetric, enzymatic assays which are important for diagnostic applications have been successfully implemented and glucose concentration measurements on several biological fluids (serum, plasma, urine, and saliva) with comparable results could be performed.[123] The microfluidic chip layout for the colorimetric glucose assay is depicted in Fig. 10, featuring reservoirs, injection structures (metering) and a network of electrodes for droplet transport, splitting and detection.

As an another example, the use of an EOWD system for the automated sample preparation of peptides and proteins for matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is reported.[130] Within this work, standard MALDI-MS reagents, analytes, concentrations, and recipes have been demonstrated to be compatible with the EWOD technology, and mass spectra comparable to those collected by conventional methods were obtained. A recent comprehensive review of the EWOD platform can be found in ref. 131.

An alternative actuation principle for surface based droplet manipulation which does not require a second electrode in contact with the droplet is based on dielectrophoresis. The basic unit operations, like droplet generation, movement, metering and merging, using this alternative actuation have been demonstrated recently.[132,133]

Surface acoustic wave driven unit operations and applications

An alternative to the electrowetting based *transport* of droplets on a plane surface has been proposed by Achim Wixforth and his group at the University of Augsburg, Germany.[134] The approach is based on surface acoustic waves (SAW) which are mechanical waves with amplitudes of typically only a few nanometres. The surface acoustic waves are generated by a

piezoelectric transducer chip (*e.g.* quartz) fabricated by placing interdigital electrodes (interdigital transducer, IDT) on top of a piezoelectric layer. Liquid droplets situated on the hydrophobic surface of the chip can be moved by the SAWs if the acoustic pressure exerted on the liquid droplet is high enough.[135] The actuation of small amounts of liquids of viscosities extending over a large range (from 1 to 1000 mPa s) has been shown.[136] This approach is also sometimes referred to as ''flat fluidics'', because no cover or slit is required unlike in the EOWD approach. Today, this technology is advanced by the company Advalytix, Germany.[137]

Metering is accomplished by placing patches of different wetting properties onto the surface. A liquid droplet is moved over a small hydrophilic ''metering spot'' *via* surface acoustic waves, leaving behind a small metered liquid portion. Also, aliquotting has been shown by moving the initial droplet over a hydrophobic/hydrophilic chessboard zone. Since the transport of a droplet depends non-linearly on the droplet size, the initial droplet of larger volume is constantly moved forward, while small, picolitre sized droplets remain on the hydrophilic parts of the wetting pattern.[134]

Mixing presents a system immanent unit operation on the SAW driven, droplet based platform. A droplet which is placed on the substrate and then gets hit by a SAW experiences an internal streaming due to the vibrating forces of the wave. If the amplitude is not large enough for droplet movement, the liquid inside the droplet is efficiently ''stirred'' while its position on the substrate is retained.

For some assay protocols, *incubation* steps at elevated temperatures are also required, *e.g.*, for a PCR amplification. Therefore, the liquid plug is placed above a micro-heating element on the substrate surface. However, since the nanolitre sized droplet possesses a high surface to volume ratio, the liquid volume decreases rapidly due to evaporation. Hence the aqueous liquid plug is covered with an oil plug having a smaller contact angle. This droplet in droplet configuration still can be moved *via* surface acoustic waves on the substrate surface. By using this technology, a PCR assay (*amplification*) within a 200 nL droplet enclosed in mineral oil was performed with an online monitoring of the DNA concentrations and provided a sensitivity of 0.1 ng.[138]

Strengths and challenges of the platform

General advantages of droplet based microfluidics are the small liquid volumes of the droplets, reducing reagent and sample consumption, thus paving the way for high throughput screening applications. Additionally, the batch mode operation scheme within the nanolitre to microlitre sized droplets represents a consistent further development of the classic assay protocols in, for example, well plates.

The pressure driven approach combines these advantages with high-throughput capabilities in a quasi continuous operational scheme. The completely enclosed liquid droplets furthermore allow the incubation and storage of liquid assay results over a long period of time without evaporation. However, the microfluidic functionality is engraved by the channel design and cannot be adopted during an assay, for example.

In contrast, the surface based actuation schemes (EWOD and SAW) come up with a high flexibility since liquid processing paths can be freely programmed. In addition, the simple setup without any moving parts can be fabricated very cost-efficiently using standard lithographic processes.

Comparing the EWOD and SAW principles for droplet actuation on the planar surface, the electrical change of the contact angle depends on the liquid properties and could cause electrolysis, while the SAW principle allows easier adaption to the liquid properties. Evaporation of liquid and the long-term stability of the hydrophobic and hydrophilic surface coatings are the major drawbacks of the surface based techniques.

VI Free scale non-contact dispensing

The free scalable non-contact dispensing platform allows delivery of liquids as free flying droplets onto planar substrates (*e.g.* microarrays), conventional containers, such as well plates, or any other target. This approach is closest to the traditional laboratory routine which is based on conducting assays *via* successive pipetting steps, manually or by automated laboratory equipment (pipetting robot). Within these fields, the dispensing of droplets of different volumes (pL to mL), from a single or up to thousands of channels in parallel, with different pitch sizes and an individual controllability is required. We focus here on the simultaneous dispensing of a large number of different reagents in parallel and disregard non-contact printing technology in general (for an overview of ink-jet printing technologies, see ref. 1). The three highlighted operating principles are all using a very similar fluidic geometry that can be fabricated based on the same fabrication technology as is described within the following section.

Unit operations on the platform

One functional unit of the free scalable non-contact liquid dispensing platform is based on the combination of a reservoir for holding the liquid, a nozzle chamber with a nozzle from which the liquid is dispensed, and a capillary channel connecting reservoir and nozzle chamber. Depending on the arrangement of these components, as well as the actuation principle, liquid volumes from several tens of picolitres to several microlitres can be dispensed. Arranging several units on a so-called dosage chip enables the dispensing of up to thousands of different liquids in parallel and has been demonstrated at a pitch ranging from several hundreds of micrometres to several millimetres. Three different actuation schemes based on the same geometrical arrangement, namely the dispensing well plate (DWP),[139,140] the TopSpot®[141,142] and the TopSpot® Vario[143] technology are depicted in Fig. 11.

The non-contact dispensing process strictly speaking represents two unit operations, liquid *metering* on the one hand, and liquid *transfer* on the other. The metered liquid volume is delivered as a free flying droplet or a jet in a non-contact manner to any substrate, receiving vessel or the reservoir of another dispensing unit. The volume of that liquid portion is determined by the nozzle geometry and the external actuation mechanism.

The DWP principle is based on the complete drainage of the liquid volume within the nozzle chamber. The micro-machined

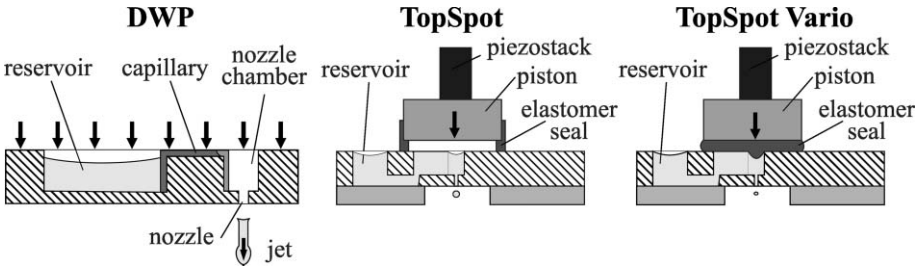

Fig. 11 DWP: pressure based actuation for dispensing from 10 nL up to several μL, TopSpot®: pressure based actuation for dispensing volumes in the lower nL range, TopSpot® Vario: direct displacement principle *via* an elastomer for dispensing of volumes from 100 pL to 1400 pL.

nozzle chamber is filled with liquid from the reservoir by capillary forces *via* the capillary channel between two dispensing events. The total liquid contained in the nozzle chamber is dispensed by applying a pneumatic pressure of 30–80 kPa for 3–10 ms. Since that pressure pulse is applied on the liquid–air interface of the nozzle chamber and the reservoir simultaneously, no pressure gradient evolves along the capillary channel and thus no backflow of liquid from the nozzle chamber to the reservoir is observed. The dosed volume is hardly affected by the liquid properties like viscosity, density and surface tension but by the geometry of the nozzle chamber only, making this dispensing method very robust.

The TopSpot® principle also relies on a pneumatic pressure pulse which, in this case, is on the sub-millisecond time scale. Such a short pneumatic pressure pulse can only be generated by compression of an enclosed gas volume. Therefore, an assembly of a piezo-stack actuator driving a piston into a closed actuation cavity above the nozzle chambers is used. The pressure pulse acts equally on all nozzle chambers within the pressurized actuation cavity and causes the simultaneous ejection of single droplets out of each nozzle. The droplet volume is typically of the order of 1 nL for a 50 μm nozzle which is, in contrast to the DWP principle, just a small fraction of the nozzle chamber volume. The exact droplet volume depends on the liquid properties, the actuation parameters and the nozzle dimensions.[144]

In contrast to the pneumatic technologies described so far, the TopSpot® Vario principle uses the direct displacement of an incompressible but easily deformable elastomer for actuation. The elastomer inlay replaces the air volume in the setup (see Fig. 11, right) and is displaced into the nozzle chambers by the piston movement if the piezo stack is actuated. A well defined volume of liquid in the nozzle chamber is displaced by the elastomer and a droplet of corresponding volume is ejected out of the nozzle. This direct displacement principle allows independent control over the droplet volume and the droplet speed. The tuneable volume of the droplets ranges from 100 pL up to 1400 pL (1.4 nL) for a 50 μm nozzle.

Also, temperature initiated *amplification* using the PCR method can be conducted within the cavities of the platform.[145] Therefore, the dispensing chip is sealed and mounted between an upper and lower heating plate, which perform a certain temperature cycle between three temperatures (94 °C, 53 °C and 72 °C). This process is similar to the common PCR cycling within standard well plates. For the microcavities within the PCR slides, however, also the upper heating plate has to change the temperature according to the cycling sequence in order to avoid temperature gradients within the PCR solution. When the cycling is finished, the PCR product (amplified DNA) can be dispensed in nanolitre portions into a microtitre plate for further processing, onto a microarray for detection or into another dispensing chip.

Application examples

One unique feature of all the three described dispensing principles is the possibility of arranging a multitude of them in parallel with a free scalable pitch of the nozzle chambers and reservoirs. The capillary channel that connects these two substructures accomplishes the format conversion from a reservoir pitch of a few millimetres (enabling the filling of the dispensing chip using standard pipetting robots) to the pitch of the nozzle of a few hundred micrometres, as required for the fabrication of microarrays for example. A DNA microarray is an ensemble of microscopic DNA spots attached to a flat solid surface forming an array of different well-known capture molecules (probes) at well-defined positions. The probes on the surface react with a complex mixture of molecules (sample) during the hybridisation phase of a microarray experiment. The sample molecules are equipped with fluorescent markers for later detection in a fluorescent readout device. One technical solution to fabricate these microarrays is based on the TopSpot® technology, a first application of the free scalable non-contact dispensing platform.[146,147]

The key advantage of the non-contact dispensing platform for microarray fabrication is the easy to perform passive format conversion within the system. The reservoirs on top of the printhead are arranged at a pitch of 2.25 mm, corresponding to the pitch of 384 well-plates, enabling filling with standard laboratory equipment, *e.g.*, pipetting robots. Several microlitres of liquids can be loaded into each of the reservoirs, which is enough for several thousand dispensing events without the need for refilling. Each reservoir is connected to a certain nozzle chamber in the middle of the printhead *via* a capillary channel (Fig. 12). The liquids are *transported* simply by capillary forces to the nozzle chamber and stop at the nozzle until a pressure pulse is applied. The nozzles are arranged in an array of 500 μm pitch on the backside of the printhead, defining the later spot positions on the microarray. The typical coefficient of variation (CV) of spot diameters on the microarray is measured to be below 1% for a single dispensing unit and smaller than 1.5% between all nozzles of a printhead for all relevant printing buffers used.[147] Using the

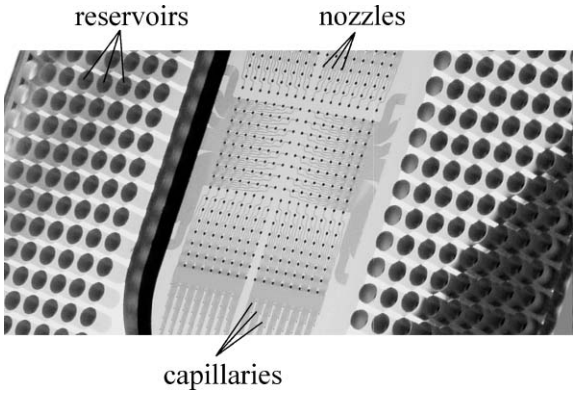

Fig. 12 The TopSpot® technology combines the unit operations reagent storage (in top side inlet reservoirs), liquid transport (through capillary channels), metering and transfer *via* a free-flying droplet through the micronozzle as soon as the piezostack is actuated. The depicted TopSpot® 384 printhead features 384 dispensing units enabling the contact-free transfer of 384 different liquids onto planar substrates (microarrays).

TopSpot® technology, also protein microarrays as well as living cell microarrays have been fabricated.[148]

A method for the simultaneous and contact-free dispensing of typically 50 nL liquid jets into micro-well plates or onto flat substrates has been realized on the dispensing platform. The so called dispensing well plate principle (Fig. 11, left) uses a set of dispensing units, arranged according to the well plate format, each featuring a liquid reservoir, a capillary channel and a nozzle chamber. All dispensing units are actuated in parallel by applying a pressure pulse on top of the dispensing chip, initiating the simultaneous and complete drainage of all nozzle chambers through the nozzle. As a result an array of individual spots is delivered to another well plate. After switching off the driving pressure, the nozzle chambers refill again from the reservoirs *via* the capillary channels. Since the reservoir contains a multiple of the nozzle chamber volume, many dispensing events can be performed before the chip has to be refilled. This, for example, enables the fast replication of a certain well plate loading into other plates (compound reformatting), or the addition of nanolitre volumes to plate based assays. The reproducibility (coefficient of variation) of the mean dosage volume has been reported to be better than 3%.[149] Based on this principle, a kinase assay based on 100 μM rhodamine substrate with a total assay volume as low as 200 nL has been demonstrated.[150]

Strengths and challenges of the platform

The wide range of dispensing volumes from 0.1 nL of the TopSpot® Vario up to 1000 nL for the DWP using the same basic geometric building blocks is certainly the main advantage of the free scalable non-contact dispensing platform. The three dispensing principles can easily be combined on one flat substrate with or without the reformatting by capillary channels and be actuated in a highly parallel mode of operation.

However, the fabrication costs of the dispensing chips have to be reduced in the future, also making disposable printheads possible to avoid laborious washing procedures. Since the cost reduction is not possible in silicon micromachining (no potential in down-scaling of the footprint of the dispensing chips or printheads) microfabrication technologies for polymers like injection moulding or hot embossing are probably the most promising alternatives. Today, this technology is pushed forward by the company BioFluidiX, Germany.[151]

Conclusion

The collection of examples of microfluidic platforms given in the previous sections shows that the platform idea has already been taken up by many groups within the microfluidics community. They do not only work on individual components fabricated in diverse technologies, but above all focus on the combination of validated fluidic unit operations by simple proved technologies. This approach allows the design and fabrication of application specific systems easily and will lead to a paradigm shift from a component and technology based research to a system oriented approach. The platforms will allow the microfluidics community to leave today's device oriented research in order to face the next challenge: the flexible and cost efficient design of hundreds to thousands of different applications that might be accessible by using the full potential of microfluidic platforms without always starting from scratch.

A good indicator of the growing interest in microfluidic platform technologies can be also seen in the remarkable number of spin-off companies that arose during recent years trying to commercialize lab-on-a-chip products based on microfluidic platform concepts. Some of them have been mentioned within this review paper, but there are many more in business already[152] or about to pop up in the near future.

References

1 H. P. Le, *J. Imaging Sci. Technol.*, 1998, **42**(1), 49–62.
2 S. C. Terry, J. H. Jerman and J. B. Angell, *IEEE Trans. Electron Devices*, 1979, **26**(12), 1880–1886.
3 A. Manz, Y. Miyahara, J. Miura, Y. Watanabe, H. Miyagi and K. Sato, *Sens. Actuators, B*, 1990, **1**(1–6), 249–255.
4 S. Shoji, M. Esashi and T. Matsuo, *Sens. Actuators*, 1988, **14**(2), 101–107.
5 H. T. G. Van Lintel, F. C. M. Vandepol and S. Bouwstra, *Sens. Actuators*, 1988, **15**(2), 153–167.
6 V. Gass, B. H. Vanderschoot, S. Jeanneret and N. F. Derooij, *Sens. Actuators, A*, 1994, **43**(1–3), 335–338.
7 P. Arquint, M. Koudelkahep, B. H. Vanderschoot, P. Vanderwal and N. F. Derooij, *Clin. Chem.*, 1994, **40**(9), 1805–1809.
8 E. Verpoorte, A. Manz, H. Ludi, A. E. Bruno, F. Maystre, B. Krattiger, H. M. Widmer, B. H. Vanderschoot and N. F. Derooij, *Sens. Actuators, B*, 1992, **6**(1–3), 66–70.
9 S. Shoji and M. Esashi, *J. Micromech. Microeng.*, 1994, **4**(4), 157–171.
10 D. J. Laser and J. G. Santiago, *J. Micromech. Microeng.*, 2004, **14**(6), R35–R64.
11 P. Woias, *Sens. Actuators, B*, 2005, **105**(1), 28–38.
12 P. Gravesen, J. Braneberg and O. S. Jensen, *J. Micromech. Microeng.*, 1993, **3**(4), 168–182.
13 N. T. Nguyen and Z. G. Wu, *J. Micromech. Microeng.*, 2005, **15**(2), R1–R16.
14 V. Hessel, H. Lowe and F. Schonfeld, *Chem. Eng. Sci.*, 2005, **60**(8–9), 2479–2501.
15 K. W. Oh and C. H. Ahn, *J. Micromech. Microeng.*, 2006, **16**(5), R13–R39.

16 G. Waibel, J. Kohnle, R. Cernosa, M. Storz, M. Schmitt, H. Ernst, H. Sandmaier, R. Zengerle and T. Strobelt, *Sens. Actuators, A*, 2003, **103**(1–2), 225–230.
17 P. S. Dittrich and A. Manz, *Nat. Rev. Drug Discovery*, 2006, **5**(3), 210–218.
18 V. Hessel and H. Lowe, *Chem. Eng. Technol.*, 2003, **26**(1), 13–24.
19 T. J. Clark, P. H. McPherson and K. F. Buechler, *Point Care*, 2002, **1**(1), 42–46.
20 P. Yager, T. Edwards, E. Fu, K. Helton, K. Nelson, M. R. Tam and B. H. Weigl, *Nature*, 2006, **442**(7101), 412–418.
21 Y.-T. Horng, P.-C. Soo, B.-J. Shen, Y.-L. Hung, K.-Y. Lo, H.-P. Su, J.-R. Wei, S.-C. Hsieh, P.-R. Hsueh and H.-C. Lai, *Water Res.*, 2006, **40**(11), 2221–2229.
22 "Micronics Inc. (www.micronics.net), Micronit Microfluidics BV (www.micronit.com), Micralyne Inc. (www.micralyne.com), i-STAT (www.abbottpointofcare.com), microfluidic ChipShop GmbH (www.microfluidic-chipshop.com), thinXXS Microtechnology AG (www.thinxxs.de)," 2007.
23 Y. N. Xia, E. Kim, X. M. Zhao, J. A. Rogers, M. Prentiss and G. M. Whitesides, *Science*, 1996, **273**(5273), 347–349.
24 Y. N. Xia and G. M. Whitesides, *Annu. Rev. Mater. Sci.*, 1998, **28**, 153–184.
25 Y. N. Xia and G. M. Whitesides, *Angew. Chem., Int. Ed. Engl.*, 1998, **37**(5), 551–575.
26 C. S. Effenhauser, G. J. M. Bruin, A. Paulus and M. Ehrat, *Anal. Chem.*, 1997, **69**(17), 3451–3457.
27 E. Delamarche, A. Bernard, H. Schmid, B. Michel and H. Biebuyck, *Science*, 1997, **276**(5313), 779–781.
28 A. Y. Fu, C. Spence, A. Scherer, F. H. Arnold and S. R. Quake, *Nat. Biotechnol.*, 1999, **17**(11), 1109–1111.
29 K. Hosokawa, T. Fujii and I. Endo, *Anal. Chem.*, 1999, **71**(20), 4781–4785.
30 D. C. Duffy, J. C. McDonald, O. J. A. Schueller and G. M. Whitesides, *Anal. Chem.*, 1998, **70**(23), 4974–4984.
31 S. K. Sia and G. M. Whitesides, *Electrophoresis*, 2003, **24**(21), 3563–3576.
32 M. A. Unger, H. P. Chou, T. Thorsen, A. Scherer and S. R. Quake, *Science*, 2000, **288**(5463), 113–116.
33 S. R. Quake and A. Scherer, *Science*, 2000, **290**(5496), 1536–1540.
34 Fluidigm Corporation, USA (www.fluidigm.com).
35 T. Thorsen, S. J. Maerkl and S. R. Quake, *Science*, 2002, **298**(5593), 580–584.
36 H. P. Chou, M. A. Unger and S. R. Quake, *Biomed. Microdevices*, 2001, **3**(4), 323–330.
37 F. R. Salemme, *Arch. Biochem. Biophys.*, 1972, **151**(2), 533–539.
38 C. L. Hansen, E. Skordalakes, J. M. Berger and S. R. Quake, *Proc. Natl Acad. Sci. U. S. A.*, 2002, **99**(26), 16531–16536.
39 C. Hansen and S. R. Quake, *Curr. Opin. Struct. Biol.*, 2003, **13**(5), 538–544.
40 J. W. Hong, V. Studer, G. Hang, W. F. Anderson and S. R. Quake, *Nat. Biotechnol.*, 2004, **22**(4), 435–439.
41 J. W. Hong and S. R. Quake, *Nat. Biotechnol.*, 2003, **21**(10), 1179–1183.
42 J. S. Marcus, W. F. Anderson and S. R. Quake, *Anal. Chem.*, 2006, **78**(9), 3084–3089.
43 J. Liu, C. Hansen and S. R. Quake, *Anal. Chem.*, 2003, **75**(18), 4718–4723.
44 J. S. Marcus, W. F. Anderson and S. R. Quake, *Anal. Chem.*, 2006, **78**(3), 956–958.
45 C. A. Burtis, N. G. Anderson, J. C. Mailen, C. D. Scott, T. O. Tiffany and W. F. Johnson, *Clin. Chem.*, 1972, **18**(8), 753.
46 N. G. Anderson, *Science*, 1969, **166**(3903), 317–324.
47 Abaxis Inc. USA (www.abaxis.com).
48 C. T. Schembri, V. Ostoich, P. J. Lingane, T. L. Burd and S. N. Buhl, *Clin. Chem.*, 1992, **38**(9), 1665–1670.
49 C. T. Schembri, T. L. Burd, A. R. Kopfsill, L. R. Shea and B. Braynin, *J. Autom. Chem.*, 1995, **17**(3), 99–104.
50 M. Madou and G. J. Kellogg, *Proc. SPIE Syst. Technol. Clin. Diagn. Drug Discovery*, 1998, **3259**, 80–93.
51 D. C. Duffy, H. L. Gillis, J. Lin, N. F. Sheppard, Jr. and G. J. Kellogg, *Anal. Chem.*, 1999, **71**(20), 4669–4678.
52 G. Ekstrand, C. Holmquist, A. E. Örlefors, B. Hellman, A. Larsson and P. Andersson, "Microfluidics in a Rotating CD," in *Proceedings of Micro Total Analysis Systems 2000*, 2000, pp. 311–314.
53 M. Madou, J. Lee, S. Daunert, S. Lai and C.-H. Shih, *Biomed. Microdevices*, 2001, **3**(3), 245–254.
54 M. Madou, J. Zoval, G. Y. Jia, H. Kido, J. Kim and N. Kim, *Annu. Rev. Biomed. Eng.*, 2006, **8**, 601–628.
55 S. Haeberle, T. Brenner, H. P. Schlosser, R. Zengerle and J. Ducrée, *Chem. Eng. Technol.*, 2005, **28**(5), 613–616.
56 SpinX Technologies, Switzerland (www.spinx-technologies.com).
57 J. Steigert, M. Grumann, T. Brenner, K. Mittenbühler, T. Nann, J. Rühe, I. Moser, S. Haeberle, L. Riegger, J. Riegler, W. Bessler, R. Zengerle and J. Ducrée, *JALA*, 2005, **10**(5), 331–341.
58 J. Steigert, M. Grumann, T. Brenner, L. Riegger, J. Harter, R. Zengerle and J. Ducrée, *Lab Chip*, 2006, **6**(8), 1040–1044.
59 C. P. Steinert, J. Mueller-Dieckmann, M. Weiss, M. Roessle, R. Zengerle and P. Koltay, "Miniaturized and Highly Parallel Protein Crystallization on a Microfluidic Disc," in *Proceedings of 19th International Conference on Micro Electro Mechanical Systems (MEMS 2007)*, Kobe, Japan, 2007, pp. 561–564.
60 J. V. Zoval and M. J. Madou, *Proc. IEEE*, 2004, **92**(1), 140–153.
61 J. Ducrée, S. Haeberle, T. Brenner, T. Glatzel and R. Zengerle, *Microfluid. Nanofluid.*, 2005, **2**(2), 97–105.
62 J. Ducrée, T. Brenner, S. Haeberle, T. Glatzel and R. Zengerle, *Microfluidics and Nanofluidics*, 2006, **2**(1), 78–84.
63 M. Grumann, A. Geipel, L. Riegger, R. Zengerle and J. Ducrée, *Lab Chip*, 2005, **5**(5), 560–565.
64 T. Brenner, T. Glatzel, R. Zengerle and J. Ducrée, *Lab Chip*, 2005, **5**(2), 146–150.
65 S. Haeberle, L. Naegele, R. Zengerle and J. Ducrée, "A Digital Centrifugal Droplet Switch For Routing of Liquids," in *Proceedings 10th International Conference on Miniaturized Systems for Chemistry and Life Sciences (μTAS 2006)*, Tokyo, Japan: 2006, pp. 570–572.
66 Gyros AB, Sweden (www.gyros.com).
67 G. Ekstrand and T. Thorsen, "Liquid Router," US Patent, US 2005/0141344 A1, 2005.
68 S. Haeberle, T. Brenner, R. Zengerle and J. Ducrée, *Lab Chip*, 2006, **6**(6), 776–781.
69 S. Lai, S. Wang, J. Luo, L. J. Lee, S. T. Yang and M. J. Madou, *Anal. Chem.*, 2004, **76**(7), 1832–1837.
70 N. Honda, U. Lindberg, P. Andersson, S. Hoffman and H. Takei, *Clin. Chem.*, 2005, **51**(10), 1955–1961.
71 M. Grumann, J. Steigert, L. Riegger, I. Moser, B. Enderle, K. Riebeseel, G. Urban, R. Zengerle and J. Ducree, *Biomed. Microdevices*, 2006, **8**(3), 209–214.
72 D. J. Harrison, A. Manz, Z. H. Fan, H. Ludi and H. M. Widmer, *Anal. Chem.*, 1992, **64**(17), 1926–1932.
73 A. Manz, D. J. Harrison, E. M. J. Verpoorte, J. C. Fettinger, A. Paulus, H. Ludi and H. M. Widmer, *J. Chromatogr.*, 1992, **593**(1–2), 253–258.
74 D. J. Harrison, K. Fluri, K. Seiler, Z. H. Fan, C. S. Effenhauser and A. Manz, *Science*, 1993, **261**(5123), 895–897.
75 C. S. Effenhauser, A. Manz and H. M. Widmer, *Anal. Chem.*, 1993, **65**(19), 2637–2642.
76 N. Sasaki, T. Kitamori and H. B. Kim, *Lab Chip*, 2006, **6**(4), 550–554.
77 C. Y. Lee, G. B. Lee, J. L. Lin, F. C. Huang and C. S. Liao, *J. Micromech. Microeng.*, 2005, **15**(6), 1215–1223.
78 D. E. Raymond, A. Manz and H. M. Widmer, *Anal. Chem.*, 1994, **66**(18), 2858–2865.
79 H. Morgan, M. P. Hughes and N. G. Green, *Biophys. J.*, 1999, **77**(1), 516–525.
80 U. Zimmermann and J. Vienken, *J. Membrane Biol.*, 1982, **67**(3), 165–182.
81 R. Krupke, F. Hennrich, H. von Lohneysen and M. M. Kappes, *Science*, 2003, **301**(5631), 344–347.
82 J. M. Ramsey, S. C. Jacobson and M. R. Knapp, *Nature Med.*, 1995, **1**(10), 1093–1096.
83 Caliper Life Sciences, USA (www.caliperls.com).
84 Agilent Technologies Inc., USA (www.agilent.com).
85 S. L. Anna, N. Bontoux and H. A. Stone, *Appl. Phys. Lett.*, 2003, **82**(3), 364–366.
86 M. Joanicot and A. Ajdari, *Science*, 2005, **309**(5736), 887–888.
87 T. Nisisako, T. Torii and T. Higuchi, *Lab Chip*, 2002, **2**(1), 24–26.
88 S. Okushima, T. Nisisako, T. Torii and T. Higuchi, *Langmuir*, 2004, **20**(23), 9905–9908.

89 A. S. Utada, E. Lorenceau, D. R. Link, P. D. Kaplan, H. A. Stone and D. A. Weitz, *Science*, 2005, **308**(5721), 537–541.
90 H. Song, J. D. Tice and R. F. Ismagilov, *Angew. Chem. Int. Ed. Engl.*, 2003, **42**(7), 768–772.
91 H. Song and R. F. Ismagilov, *J. Am. Chem. Soc.*, 2003, **125**(47), 14613–14619.
92 B. Zheng, J. D. Tice and R. F. Ismagilov, *Anal. Chem.*, 2004, **76**(17), 4977–4982.
93 I. Shestopalov, J. D. Tice and R. F. Ismagilov, *Lab Chip*, 2004, **4**(4), 316–321.
94 M. Y. He, J. S. Edgar, G. D. M. Jeffries, R. M. Lorenz, J. P. Shelby and D. T. Chiu, *Anal. Chem.*, 2005, **77**(6), 1539–1544.
95 D. R. Link, S. L. Anna, D. A. Weitz and H. A. Stone, *Phys. Rev. Lett.*, 2004, **92**(5), 054503.
96 J. D. Tice, H. Song, A. D. Lyon and R. F. Ismagilov, *Langmuir*, 2003, **19**(22), 9127–9133.
97 J. D. Tice, A. D. Lyon and R. F. Ismagilov, *Anal. Chim. Acta*, 2004, **507**(1), 73–77.
98 H. Song, M. R. Bringer, J. D. Tice, C. J. Gerdts and R. F. Ismagilov, *Appl. Phys. Lett.*, 2003, **83**(22), 4664–4666.
99 M. R. Bringer, C. J. Gerdts, H. Song, J. D. Tice and R. F. Ismagilov, *Phil. Trans. R. Soc. London, Ser. A*, 2004, **362**(1818), 1087–1104.
100 A. Gunther, S. A. Khan, M. Thalmann, F. Trachsel and K. F. Jensen, *Lab Chip*, 2004, **4**(4), 278–286.
101 A. Gunther, M. Jhunjhunwala, M. Thalmann, M. A. Schmidt and K. F. Jensen, *Langmuir*, 2005, **21**(4), 1547–1555.
102 S. A. Khan, A. Gunther, M. A. Schmidt and K. F. Jensen, *Langmuir*, 2004, **20**(20), 8604–8611.
103 P. Garstecki, M. A. Fischbach and G. M. Whitesides, *Appl. Phys. Lett.*, 2005, **86**(24), 244108.
104 K. Martin, T. Henkel, V. Baier, A. Grodrian, T. Schon, M. Roth, J. M. Kohler and J. Metze, *Lab Chip*, 2003, **3**(3), 202–207.
105 D. R. Link, E. Grasland-Mongrain, A. Duri, F. Sarrazin, Z. D. Cheng, G. Cristobal, M. Marquez and D. A. Weitz, *Angew. Chem., Int. Ed. Engl.*, 2006, **45**(16), 2556–2560.
106 K. Ahn, C. Kerbage, T. P. Hunt, R. M. Westervelt, D. R. Link and D. A. Weitz, *Appl. Phys. Lett.*, 2006, **88**(2), 024104.
107 Raindance Technologies, USA (*www.raindancetechnologies.com*).
108 B. Zheng, L. S. Roach and R. F. Ismagilov, *J. Am. Chem. Soc.*, 2003, **125**(37), 11170–11171.
109 B. Zheng, J. D. Tice, L. S. Roach and R. F. Ismagilov, *Angew. Chem., Int. Ed. Engl.*, 2004, **43**(19), 2508–2511.
110 M. K. Yadav, C. J. Gerdts, R. Sanishvili, W. W. Smith, L. S. Roach, R. F. Ismagilov, P. Kuhn and R. C. Stevens, *J. Appl. Crystallogr.*, 2005, **38**, 900–905.
111 L. S. Roach, H. Song and R. F. Ismagilov, *Anal. Chem.*, 2005, **77**(3), 785–796.
112 D. L. Chen, C. J. Gerdts and R. F. Ismagilov, *J. Am. Chem. Soc.*, 2005, **127**(27), 9672–9673.
113 B. Zheng, C. J. Gerdts and R. F. Ismagilov, *Curr. Opin. Struct. Biol.*, 2005, **15**(5), 548–555.
114 B. Zheng, J. D. Tice and R. F. Ismagilov, *Adv. Mater.*, 2004, **16**(15), 1365–1368.
115 B. Zheng and R. F. Ismagilov, *Angew. Chem., Int. Ed. Engl.*, 2005, **44**(17), 2520–2523.
116 D. L. L. Chen and R. F. Ismagilov, *Curr. Opin. Chem. Biol.*, 2006, **10**(3), 226–231.
117 F. Mugele and J. C. Baret, *J. Phys.: Condens. Matter*, 2005, **17**(28), R705–R774.
118 J. Lee, H. Moon, J. Fowler, T. Schoellhammer and C. J. Kim, *Sens. Actuators, A*, 2002, **95**(2–3), 259–268.
119 F. Mugele, A. Klingner, J. Buehrle, D. Steinhauser and S. Herminghaus, *J. Phys.: Condens. Matter*, 2005, **17**(9), S559–S576.
120 M. G. Pollack, R. B. Fair and A. D. Shenderov, *Appl. Phys. Lett.*, 2000, **77**(11), 1725–1726.
121 J. Lee and C. J. Kim, *J. Microelectromech. Syst.*, 2000, **9**(2), 171–180.
122 D. Chatterjee, B. Hetayothin, A. R. Wheeler, D. J. King and R. L. Garrell, *Lab Chip*, 2006, **6**(2), 199–206.
123 V. Srinivasan, V. K. Pamula and R. B. Fair, *Lab Chip*, 2004, **4**(4), 310–315.
124 H. Ren, R. B. Fair and M. G. Pollack, *Sens. Actuators, B*, 2004, **98**(2–3), 319–327.
125 J. Berthier, P. Clementz, O. Raccurt, D. Jary, P. Claustre, C. Peponnet and Y. Fouillet, *Sens. Actuators, A*, 2006, **127**(2), 283–294.
126 M. G. Pollack, A. D. Shenderov and R. B. Fair, *Lab Chip*, 2002, **2**(2), 96–101.
127 S. K. Cho, H. J. Moon and C. J. Kim, *J. Microelectromech. Syst.*, 2003, **12**(1), 70–80.
128 P. Paik, V. K. Pamula, M. G. Pollack and R. B. Fair, *Lab Chip*, 2003, **3**(1), 28–33.
129 P. Paik, V. K. Pamula and R. B. Fair, *Lab Chip*, 2003, **3**(4), 253–259.
130 A. R. Wheeler, H. Moon, C. J. Kim, J. A. Loo and R. L. Garrell, *Anal. Chem.*, 2004, **76**(16), 4833–4838.
131 R. B. Fair, *Microfluid. Nanofluid.*, 2007, **3**(3), 245–281.
132 P. R. C. Gascoyne, J. V. Vykoukal, J. A. Schwartz, T. J. Anderson, D. M. Vykoukal, K. W. Current, C. McConaghy, F. F. Becker and C. Andrews, *Lab Chip*, 2004, **4**(4), 299–309.
133 J. A. Schwartz, J. V. Vykoukal and P. R. C. Gascoyne, *Lab Chip*, 2004, **4**(1), 11–17.
134 A. Wixforth, *Superlattices Microstruct.*, 2003, **33**(5–6), 389–396.
135 A. Wixforth, C. Strobl, C. Gauer, A. Toegl, J. Scriba and Z. von Guttenberg, *Anal. Bioanal. Chem.*, 2004, **379**(7–8), 982–991.
136 D. Beyssen, L. Le Brizoual, O. Elmazria and P. Alnot, *Sens. Actuators, B*, 2006, **118**(1–2), 380–385.
137 Advalytix AG, Germany (www.advalytix.de).
138 Z. Guttenberg, H. Muller, H. Habermuller, A. Geisbauer, J. Pipper, J. Felbel, M. Kielpinski, J. Scriba and A. Wixforth, *Lab Chip*, 2005, **5**(3), 308–317.
139 P. Koltay, R. Steger, B. Bohl and R. Zengerle, *Sens. Actuators, A*, 2004, **116**(3), 483–491.
140 P. Koltay, J. Kalix and R. Zengerle, *Sens. Actuators, A*, 2004, **116**(3), 472–482.
141 B. de Heij, C. Steinert, H. Sandmaier and R. Zengerle, *Sens. Actuators, A*, 2003, **103**(1–2), 88–92.
142 B. de Heij, M. Daub, O. Gutmann, R. Niekrawietz, H. Sandmaier and R. Zengerle, *Anal. Bioanal. Chem.*, 2004, **378**(1), 119–122.
143 C. P. Steinert, I. Goutier, O. Gutmann, H. Sandmaier, M. Daub, B. de Heij and R. Zengerle, *Sens. Actuators, A*, 2004, **116**(1), 171–177.
144 O. Gutmann, R. Niekrawietz, R. Kuehlewein, C. P. Steinert, B. de Heij, R. Zengerle and M. Daub, *Sens. Actuators, A*, 2004, **116**(2), 187–194.
145 R. M. Kaack, A. Jung, M. H. Wenz, R. Zengerle and M. Daub, ''PCR-slide: A modular and cascadable platform for DNA sample processing with integrated nanolitre dosage,'' in *Proceedings of IEEE-MEMS 2004*, 2004.
146 O. Gutmann, R. Niekrawietz, R. Kuehlewein, C. P. Steinert, S. Reinbold, B. de Heij, M. Daub and R. Zengerle, *Analyst*, 2004, **129**(9), 835–840.
147 O. Gutmann, R. Kuehlewein, S. Reinbold, R. Niekrawietz, C. P. Steinert, B. de Heij, R. Zengerle and M. Daub, *Biomed. Microdevices*, 2004, **6**(2), 131–137.
148 O. Gutmann, R. Kuehlewein, S. Reinbold, R. Niekrawietz, C. P. Steinert, B. de Heij, R. Zengerle and M. Daub, *Lab Chip*, 2005, **5**(6), 675–681.
149 R. Steger, B. Bohl, R. Zengerle and P. Koltay, *J. Assoc. Lab. Autom.*, 2004, **9**(5), 291–299.
150 R. Steger, C. Mehne, N. Wangler, M. Heckele, R. Zengerle and P. Koltay, ''Drop in Drop Nanoliter Kinase Assay Made with Hot Embossed Disposable Multi Channel Dispenser,'' in *Proceedings 10th International Conference on Miniaturized Systems for Chemistry and Life Sciences (μTAS 2006)*, Tokyo, Japan: 2006, pp. 999–1001.
151 BioFluidiX GmbH, Germany (www.biofluidix.com).
152 C. Haber, *Lab Chip*, 2006, **6**(9), 1118–1121.

