Microfluidic Platforms for Miniaturization, Integration and Automation of Biochemical Assays

Dipl. Ing. Stefan Haeberle[1], PD Dr. Jens Ducrée[1], Dr. Felix von Stetten[2], Prof. Dr. Roland Zengerle[1, 2]

[1] Institut für Mikro- und Informationstechnik der Hahn-Schickard-Gesellschaft (HSG-IMIT), Wilhelm-Schickard-Straße 10, 78052 Villingen-Schwenningen
[2] Universität Freiburg, Institut für Mikrosystemtechnik (IMTEK), Lehrstuhl für Anwendungsentwicklung, Georges-Koehler-Allee 106, 79110 Freiburg

Summary

In this contribution we discuss two microfluidic platforms that enable the miniaturization, integration and automation of biochemical assays. The first platform, known as "capillary test stripes" or also as "lateral flow assays", can be regarded as the gold-standard of today's point-of-care diagnostics. The second platform, "centrifugal microfluidics", is under development in our lab. It allows enhancing the accuracy of assays and enables easy parallelisation. Both platforms allow to perform a set of microfluidic functions – defined as microfluidic unit operations – which can be easily combined within a well defined and consistent fabrication technology. This way application specific biochemical assays can be implemented in a flexible and cost-efficient way.

1 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 a consumer wants to buy itself. 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 industry.

The history of microfluidics dates back to the early 1950s when efforts to dispense small amounts of liquids in the nano- and subnanoliter range were made for providing the basics of today's ink-jet technology [1]. In terms of fluid propulsion within microchannels of submillimeters 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 microvalves [4] and micropumps [5;6] based on silicon micromachining were also 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.

Following these pioneer workings, thousands of researchers spent a lot of time to develop 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 micropumps have been published [9-12], many different types of mixers [13;14], 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 if we really need more of those components? Due to 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 with 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. 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 [16]). 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.

microfluidic *unit operations*	**fabrication technology**
• fluid transport • fluid valving • fluid mixing • separation • concentration / amplification / accumulation • detection / readout • reagent storage • incubation • …	validated manufacturing technology for the whole set of fluidic ***unit operations*** (prototyping and mass fabrication) **seamless integration of different elements** • preferable in a monolithic way • or by a cheap and simple packaging technique

Table 1:
Common features of microfluidic platforms.

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 to other platforms. Thus speaking about microfluidic platforms involves also at least one validated fabrication technology to realize complete systems out of the elements. This results in a definition of a microfluidic platform as follows.

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 to implement different application specific systems (assays) in an easy and flexible way, based on the same fabrication technology.

In this paper we start with reviewing the working principle of "capillary test stripes". They define the state of the art in today's point-of-care diagnostics and can be regarded as the gold standard all lab-on-a-chip systems have to compete with. After that we will discuss the "centrifugal microfluidics" platform that is under development in our lab.

2 Capillary Driven Test Strips

Test strips or "lateral flow assays" as they are also called, are well known in the diagnostic field since the 1960s representing the "state-of-the-art" with billions of units that are produced at 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 from a microfluidic point of view exists. And this although the complexity of test strips varies from a single fleece for e.g. pH measurement to very complex and partially also microstructured configurations of multiple fleeces that enable the implementation of more complex tests like immunoassays.

2.1 Unit Operations

The basic principle of the platform is the 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 locally pricked with a lancet before. 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 Figure 1. The separation fleece is placed directly underneath the start reservoir into which the blood sample is applied.

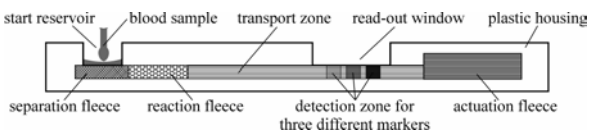

Figure 1 Simplified cross section of a typical capillary driven immunoassay test strip.

Typically, ***reagent storage*** is done in terms of dried reagents that have been pre-deposited into the fleeces during fabrication. ***Dissolving*** of 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 microchamber featuring e.g. 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 done by the defined volumes of the fleeces and microstructures. The liquid flow stops automatically, as soon as the actuation fleece (Figure 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.

The results from a test strip assay are mostly readout by optical markers. Since the concentration of those molecules 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 color it. However, only clear and binary signal generating assays such as pregnancy tests are capable for the manual readout. Some assays are also readout using electrochemical mechanisms. The glucose concentration of a blood sample is determined by measuring the electrical charge generated during the enzymatical oxidation of glucose to gluconic acid for example. The test strip reader applies and external electric potential and measures the current which is a function of the generated numbers of electrons.

2.2 Application Examples

A huge amount of assays have been developed on the capillary test strip platform during the past forty years and are mainly published in clinical diagnostics and immunological journals. Here, the reader shall only be sensitized to not loose 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 pure disposable test carrier, that do not need any electricity for carrying out the test and can be readout visually are predestinated 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 of running an agarose gel after the standard PCR have been shown [21]. The multiplex-nested PCR is performed within a standard thermal cycler and the results are subsequently readout in a lateral flow assay via colloidal gold labeling and visual inspection. This makes the complex and error-prone readout via running an agarose gel obsolete.

2.3 Strength and Challenges of the Platform

The possibility to perform 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. No external energy is required which opens up a wide field of applications especially for simple binary tests but 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.

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 can not be influenced actively, once the process is started. As a consequence the exact timing of assay steps depends on variations on viscosity and surface tension of the sample. Other

crucial unit operations are metering and incubation whose accuracy 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 the 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 behaviors. 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.

3. Centrifugal Microfluidics

The approach of using centrifugal forces to process samples and reagents dates back to the end of the 1960s [22;23]. At that time, centrifugal analyzers have 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. In the beginning of the 1990s, the company Abaxis [24] developed the portable clinical chemistry analyzer [25;26]. 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.

A next generation of centrifugal devices emerged from the technical capabilities offered by microfabrication and microfluidic technologies [27-30]. Length scales of the fluidic structures in the range of a few hundred micrometers allow parallel processing of up to 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 on the theory and applications on the centrifugal microfluidic platform has been published recently [31].

3.1 Unit Operations

Liquid transport is initiated by the radial outwards directed centrifugal force f_w which can be scaled over a wide range by the frequency of rotation w together with the flow resistance of the fluidic channels. Small flow rates in the order of nL/s as well as high throughput continuous flows up to 1 mL / s [32] can be generated. So scaling of flow rates over 6 orders of magnitude and independent from 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 be realized by three different microfluidic structures on the centrifugal platform, as depicted in Figure 2. A very simple valve arises at the sudden expansion of a microfluidic channel e.g. into a bigger reservoir. The valving mechanism of this capillary valve is based on the energy barrier for the proceeding 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 [28]. 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 w_c can be attributed to every valve structure. Another possibility to stop the liquid flow within a channel is the locally hydrophobic coating of the channel walls (Figure 2, B). Also this valve is opened as soon as the rotational frequency exceeds the critical burst frequency w_c. A third method is based on a hydrophilic U-shaped syphon channel, wherein the two liquid-gas interfaces are leveraged at high frequencies of rotation (Figure 2, C). Below a critical frequency w_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 syphon.

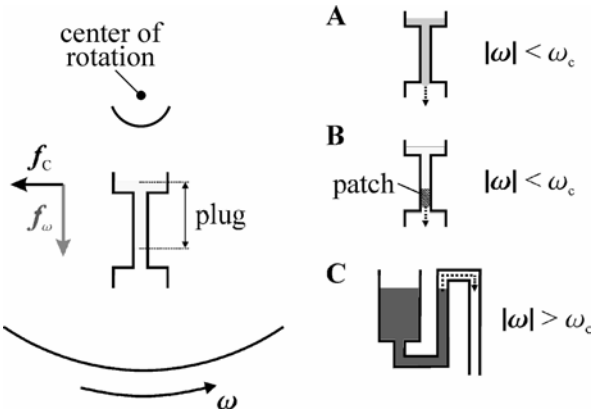

Figure 2 Principal centrifugal approach and schematic sketch of the three valving techniques on the centrifugal platform. (A) Geometric capillary valve, (B) hydrophobic valve and (C) hydrophilic syphon valve.

An alternative approach for the control of liquid flows on the centrifugal platform is followed by the company Spin-X technologies, Switzerland [33]. 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. Due to that, the Spin-X platform works with a standardized fluidic cartridge that is not custom made for each specific application, but can be programmed right 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 [34]. The metered liquid portion is directly set by the volume capacity of the chamber. Due to the high precision of micro-fabrication technologies, small coefficients of variations (standard deviation / mean value), e.g. a CV < 5% for a volume of 300 nL [35] and also metered volumes of 5 nanoliters only have been achieved [36]. By arranging several metering structures interconnected via an appropriate distribution channel, simple aliquotting structures can be realized [37]. 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 radial directed rotating channel, the perpendicular directed Coriolis force automatically generates a transversal liquid flow [32;38;39]. 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 per channel [32]. Besides the mixing of continuous liquid flows, also the homogenization of discrete and small liquid volumes located in chambers is 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) [34]. This method leads to reduced mixing times in the order of several seconds compared to 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 [40].

For routing (***switching***) of liquids, a switch availing the transversal Coriolis force to guide liquid flows between two outlets at the branching of an inverse Y-shaped channel has been presented [41]. 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 only at low frequencies down to a few Hz [42]. Another method for liquid-routing based on different wetting properties of the continuative channels has been reported by Gyros AB, Sweden [43]. 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 [44].

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 [26]. 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 [45]. 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 20 s only.

3.2 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 [46]. 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-wellplate, while having advantages over the conventional method such as less reagent consumption and shorter assay time.

Gyros AB, Sweden [43] uses a flow-through sandwich immunoassay at nanoliter scale to quantify proteins within the Gyrolab™ Workstation. Therefore, a column of pre-packed and streptavidin coated microparticles 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) imprecision (CV) of the immunoassays on the microfluidic disk are below 20 % [47]. 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 microliters.

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 [35]. After loading the reagents into the reagents reservoir, a droplet of untreated human blood derived from 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 seconds 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 [48]. 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 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 Figure 3 [36]. First, a defined volume of the protein solution is dispensed into the protein inlet and transported into the crystallization chamber. Afterwards, the preloaded precipitant is metered under rotation and transferred into the crystallization chamber as soon as the hydrophobic valve breaks. In the last step, the preloaded 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.

3.3 Strength 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 be easily combined in a monolithic way within the same fabrication process. Those elements allow implementing all needed unit operations to perform complex assay protocols in an automated way. Due 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.

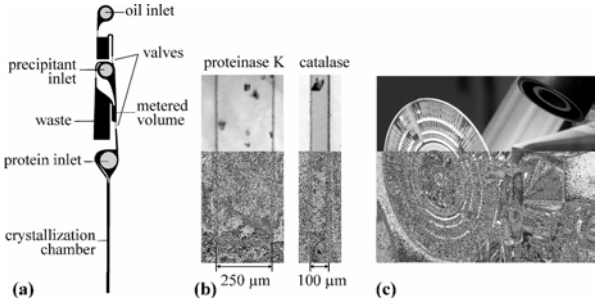

Figure 3 Microfluidic realization of a free interface diffusion (FID) protein crystallization assay, based on the centrifugal microfluidic platform (a) [36]. The three liquids (protein, metered precipitant, oil) are subsequently transported into the crystallization chamber triggered by hydrophobic valves. 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).

As soon as any additional actuation or sensing function is required on the module while rotating, things however become tricky from a technical point of view if a contact free interfacing is not applicable. The platform also lacks of flexibility compared to others that allow online 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.

4. Conclusions

The two examples of microfluidic platforms given in the previous sections show 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 to design and fabricate application specific systems easily and will lead to a paradigm shift from a component 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 starting always from scratch.

A good indicator for the growing interest in microfluidic platform technologies can be also seen in the remarkable number of spin-off companies that arose during the last 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 much more in business already [49] or will pop up in the near future.

5. References

[1] Le, H. P., *Journal of Imaging Science and Technology*, **42,** 49-62, 1998.

[2] Terry, S. C., Jerman, J. H., and Angell, J. B., *Ieee Transactions on Electron Devices*, **26,** 1880-1886, 1979.

[3] Manz, A., Miyahara, Y., Miura, J., Watanabe, Y., Miyagi, H., and Sato, K., *Sensors and Actuators B-Chemical*, **1,** 249-255, 1990.

[4] Shoji, S., Esashi, M., and Matsuo, T., *Sensors and Actuators*, **14,** 101-107, 1988.

[5] Van Lintel, H. T. G., Vandepol, F. C. M., and Bouwstra, S., *Sensors and Actuators*, **15,** 153-167, 1988.

[6] Gass, V., Vanderschoot, B. H., Jeanneret, S., and Derooij, N. F., *Sensors and Actuators A-Physical*, **43,** 335-338, 1994.

[7] Arquint, P., Koudelkahep, M., Vanderschoot, B. H., Vanderwal, P., and Derooij, N. F., *Clinical Chemistry*, **40,** 1805-1809, 1994.

[8] Verpoorte, E., Manz, A., Ludi, H., Bruno, A. E., Maystre, F., Krattiger, B., Widmer, H. M., Vanderschoot, B. H., and Derooij, N. F., *Sensors and Actuators B-Chemical*, **6,** 66-70, 1992.

[9] Shoji, S. and Esashi, M., *Journal of Micromechanics and Microengineering*, **4,** 157-171, 1994.

[10] Laser, D. J. and Santiago, J. G., *Journal of Micromechanics and Microengineering*, **14,** R35-R64, 2004.

[11] Woias, P., *Sensors and Actuators B-Chemical*, **105,** 28-38, 14-2-2005.

[12] Gravesen, P., Braneberg, J., and Jensen, O. S., *Journal of Micromechanics and Microengineering*, **3,** 168-182, 1993.

[13] Nguyen, N. T. and Wu, Z. G., *Journal of Micromechanics and Microengineering*, **15,** R1-R16, 2005.

[14] Hessel, V., Lowe, H., and Schonfeld, F., *Chemical Engineering Science*, **60,** 2479-2501, 2005.

[15] Oh, K. W. and Ahn, C. H., *Journal of Micromechanics and Microengineering*, **16,** R13-R39, 2006.

[16] Haeberle, S. and Zengerle, R., *Lab Chip*, online first-DOI: 10.1039/b706364b, 2007.

[17] Dittrich, P. S. and Manz, A., *Nature Reviews Drug Discovery*, **5,** 210-218, 2006.

[18] Hessel, V. and Lowe, H., *Chemical Engineering & Technology*, **26,** 13-24, 2003.

[19] Clark, T. J., McPherson, P. H., and Buechler, K. F., *Point of Care*, **1,** 42-46, 2002.

[20] Yager, P., Edwards, T., Fu, E., Helton, K., Nelson, K., Tam, M. R., and Weigl, B. H., *Nature*, **442,** 412-418, 27-7-2006.

[21] Horng, Y-T., Soo, P-C., Shen, B-J., Hung, Y-L., Lo, K-Y., Su, H-P., Wei, J-R, Hsieh, S-C., Hsueh, P-R., and Lai, H-C., *Water Research*, **40,** 2221-2229, 11-6-2006.

[22] Burtis, C. A., Anderson, N. G., Mailen, J. C., Scott, C. D., Tiffany, T. O., and Johnson, W. F., *Clinical Chemistry*, **18,** 753-&, 1972.

[23] Anderson, N. G., *Science*, **166,** 317-324, 1969.

[24] Abaxis Inc. USA, www.abaxis.com, accessed 2007.

[25] Schembri, C. T., Ostoich, V., Lingane, P. J., Burd, T. L., and Buhl, S. N., *Clin.Chem.*, **38,** 1665-1670, 1992.

[26] Schembri, C. T., Burd, T. L., Kopfsill, A. R., Shea, L. R., and Braynin, B., *Journal of Automatic Chemistry*, **17,** 99-104, 1995.

[27] Madou, Marc and Kellogg, Gregory J., *Proc.SPIE Systems and Technologies for Clinical Diagnostics and Drug Discovery*, **3259,** 80-93, 1998.

[28] Duffy, David C., Gillis, Heather L., Lin, Joe, Sheppard, Norman F., Jr., and Kellogg, Gregory J., *Analytical Chemistry*, **71,** 4669-4678, 1999.

[29] Ekstrand, Gunnar, Holmquist, Claes, Örlefors, Anna Edmann, Hellman, Bo, Larsson, Anders, and Andersson, Per, *Proceedings of Micro Total Analysis Systems 2000*, 311-314, 2000.

[30] Madou, Marc, Lee, James, Daunert, Sylvia, Lai, Siyi, and Shih, Chih-Hsin, *Biomedical Microdevices*, **3,** 245-254, 2001.

[31] Madou, M., Zoval, J., Jia, G. Y., Kido, H., Kim, J., and Kim, N., *Annual Review of Biomedical Engineering*, **8,** 601-628, 2006.

[32] Haeberle, S., Brenner, T., Schlosser, H. P., Zengerle, R., and Ducrée, J., *Chemical Engineering & Technology*, **28,** 613-616, 2005.

[33] SpinX Technologies, Switzerland, www.spinx-technologies.com, accessed 2007.

[34] Steigert, J., Grumann, M., Brenner, T., Mittenbühler, K., Nann, T., Rühe, J, Moser, I., Haeberle, S., Riegger, L., Riegler, J., Bessler, W., Zengerle, R., and Ducrée, J., *Journal of the Association for Laboratory Automation (JALA)*, **10,** 331-341, 2005.

[35] Steigert, J., Grumann, M., Brenner, T, Riegger, L., Harter, J., Zengerle, R., and Ducrée, J., *Lab Chip*, **6,** 1040-1044, 2006.

[36] Steinert, C. P., Mueller-Dieckmann, J., Weiss, M., Roessle, M., Zengerle, R., and Koltay, P., *Proceedings of 19th International Conference on Micro Electro Mechanical Systems (MEMS 2007)*, 561-564, 2007.

[37] Zoval, J. V. and Madou, M. J., *Proceedings of the IEEE*, **92,** 140-153, 2004.

[38] Ducrée, J., Haeberle, S., Brenner, T, Glatzel, T., and Zengerle, R., *Microfluidics and Nanofluidics*, **2,** 97-105, 2005.

[39] Ducrée, J., Brenner, T, Haeberle, S., Glatzel, T., and Zengerle, R., *Microfluidics and Nanofluidics*, **2,** 78-84, 2006.

[40] Grumann, M., Geipel, A., Riegger, L., Zengerle, R., and Ducrée, J., *Lab on A Chip*, **5,** 560-565, 2005.

[41] Brenner, T., Glatzel, T., Zengerle, R., and Ducrée, J., *Lab on A Chip*, **5,** 146-150, 2005.

[42] Haeberle, S., Naegele, L., Zengerle, R., and Ducrée, J., *Proceedings 10th International Conference on Miniaturized Systems for Chemistry and Life Sciences (μTAS 2006)*, 570-572, 2006.

[43] Gyros AB, Sweden, www.gyros.com, accessed 2007.

[44] Ekstrand, G. and Thorsen, T., US 2005/0141344 A1, 2005.

[45] Haeberle, S., Brenner, T., Zengerle, R., and Ducrée, J., *Lab on A Chip*, **6,** 776-781, 2006.

[46] Lai, S., Wang, S., Luo, J., Lee, L. J., Yang, S. T., and Madou, M. J., *Anal.Chem.*, **76,** 1832-1837, 1-4-2004.

[47] Honda, N., Lindberg, U., Andersson, P., Hoffman, S., and Takei, H., *Clinical Chemistry*, **51,** 1955-1961, 2005.

[48] Grumann, M., Steigert, J., Riegger, L., Moser, I., Enderle, B., Riebeseel, K., Urban, G., Zengerle, R., and Ducree, J., *Biomedical Microdevices*, **8,** 209-214, 2006.

[49] Haber, C., *Lab on A Chip*, **6,** 1118-1121, 2006.