

## Microfluidic Platforms for Miniaturization, Integration and Automation of Biochemical Assays

T. Metz<sup>a</sup>, D. Mark<sup>a</sup>, S. Häberle<sup>a</sup>, G. Roth<sup>b</sup>, C. Müller<sup>c</sup>, F. von Stetten<sup>a</sup>, R. Zengerle<sup>a,b</sup>

<sup>a</sup> HSG-IMIT – Institute for Micromachining and Information Technology,  
Wilhelm-Schickard-Straße 10, 78052 Villingen-Schwenningen, Germany

<sup>b</sup> Laboratory for MEMS Applications, Department of Microsystems Engineering (IMTEK),  
University of Freiburg, Georges-Koehler-Allee 106, 79110, Freiburg, Germany

<sup>c</sup> Laboratory for Process Technologies, Department of Microsystems Engineering (IMTEK),  
University of Freiburg, Georges-Koehler-Allee 106, 79110, Freiburg, Germany

### Abstract

During the last decade many technological progresses were achieved in the field of Lab-on-a-Chip for the miniaturization, integration and automation of biochemical assays. Nowadays an unmanageable variety of alternative approaches exist that can perform these tasks. To cope with the complexity of those systems the paradigm of development changed from component oriented approaches towards the development of microfluidic platforms. 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 implementation of different application specific systems (assays) in an easy and flexible way, based on the same fabrication technology. In this work we will discuss important issues for the successful implementation of a platform oriented development environment for Lab-on-a-Chip on the example of the newly set up Lab-on-a-Chip Foundry Service of the HSG-IMIT. The systematic development of Lab-on-a-Chip will be shown on the example of a nucleic acid assay on the centrifugal microfluidic platform. In the Lab-on-a-Chip Foundry Service, development is based on standardized microfluidics and standardized fabrication processes that are maintained in a knowledge management system, the Lab-on-a-Chip Design Handbook of the HSG-IMIT. Facilities and experts are provided for microfabrication and microfluidics as well as for biochemistry and biology to enable fast development cycles in a one-stop shop approach. Prototyping is organized in rapid prototyping chains for polymer fabrication, sealing and assembly in a rapid prototyping workshop.

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

### 1. Microfluidic platforms for the development of microfluidic systems

During the last decade a paradigm change in the development of microfluidic systems has been performed throughout the community. While the earliest markets for microfluidic systems were given by single solution systems like inkjet printheads, micropumps or microvalves the emerging field of Lab-on-a-Chip requires the integration of multiple fluidic components onto a single system with the ability of a well defined fabrication process to fabricate cost efficient devices.

Due to the large diversity in microfluidic developments, collaborations and supply chains of different companies forming new products by just combining off-the-shelf components are still out of reach. This lack of standards and defined interfaces is at least one bottleneck for the commercial proliferation of microfluidic technologies.

In contrast, microfluidic platforms provide a variety of design components and fabrication technologies that are well tuned onto each other [1]. This enables the development of Lab-on-a-Chip systems where complex biochemical assays that commonly require multiple laboratory tasks employing multiple devices are integrated and automatized on a single miniaturized system. In Fig. 1 the integration of a complex laboratory protocol on a single device using only one passive disposable in form of a fluidic disk is sketched.

Market expectations for Lab-on-a-Chip systems are high [2] as there are a number of applications and potential benefits of such systems. E.g. in the diagnostic market many highly complex tests should be possible to be performed at the point of care using mobile Lab-on-a-Chip what could significantly improve the medical quality and efficiency. Nevertheless, while some products were successfully brought into the market like the pressure driven I-Stat® for clinical point of care diagnostics from the US Company Abbot [3] or the veterinary diagnostic system from ABAXIS [4] a breakthrough of Lab-on-a-Chip is still not achieved.

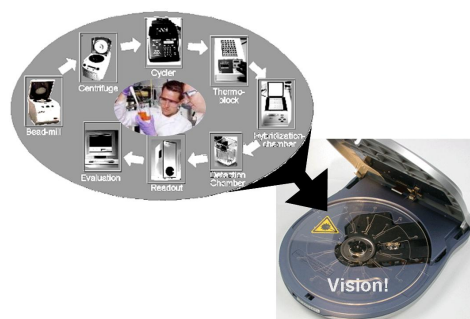


Fig. 1. Integration of a complex laboratory protocol.

In particular the stated complexity in microfluidic development that is set on top of the already complex biochemical assay development increases the development risks and the time to market of Lab-on-a-Chip.

A systematic workflow on established microfluidic platforms is necessary to make the development of Lab-on-a-Chip more efficient, while being still flexible enough to allow the miniaturization and automation of many biochemical assays.

Typical microfluidic platforms that are valid for a platform oriented development are capillary test stripes 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 or SAW driven microfluidics. Nevertheless more platforms or platform type systems are available.

In a microfluidic platform basic fluidic unit operations required within a given application area must be flexible realized to be adaptable to different assays. Such basic fluidic unit operations are fluid transport, fluid metering, fluid mixing, valving, and separation or concentration of molecules or particles. The collection of fluidic unit operations needed for diagnostic applications may have only little overlap with the collection needed for pharmaceutical applications [5] or for applications in micro-reaction technology [6]. 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.

In this work we will focus on the centrifugal microfluidic platform and show our approach to implement a systematic workflow for the implementation of arbitrary state of the art biochemical assays.

## 2. Systematic implementation of assays

Implementation of an assay into the centrifugal microfluidic platform is achieved by a systematic workflow in four steps as shown in Fig. 2.

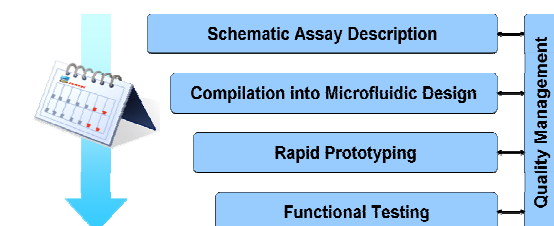


Fig. 2. Assay implementation scheme in four steps.

First, the assay is divided into laboratory unit operations (LUO's) such as pipetting steps, mixing steps, thermocycling or readout steps which are commonly realized on different devices with a necessary transfer of samples between. An example of such a description is shown in Fig. 3 for a PCR based DNA analysis assay including a pre-amplification step.

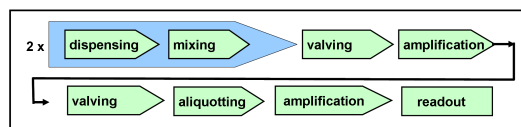


Fig. 3 Representation of Assay by LUO's.

Second the LUO's are compiled into a layout and device protocol of microfluidic unit operations according to given rules. In Fig. 4 the compilation of the previously shown assay into the microfluidic design for the centrifugal microfluidic platform is shown.

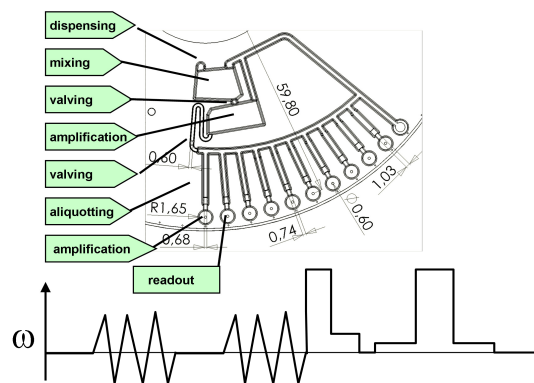


Fig. 4 Microfluidic design implementing all LUO's and the necessary frequency protocol.

This design was realized in our prototyping workshop by thermoforming of with the resulting cartridge shown in Fig. 5 and can be used on the commercially available centrifugal thermocycler Rotorgene 2000®.

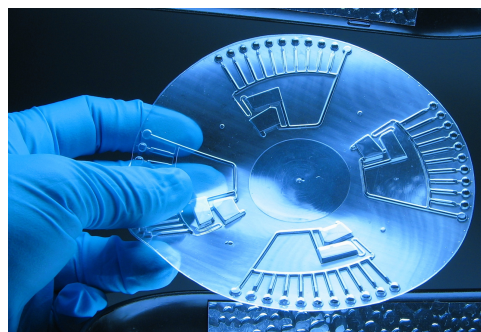


Fig. 5 Fabricated test structure by thermoforming of foils.

Using this device, PCR experiments were already successfully performed using such blister cartridges. Results are shown in Fig. 6.

At the HSG-IMIT and IMTEK Lab-on-a-Chip group a design handbook with parameterized and validated unit operations for different microfluidic platforms and fabrication processes is set up. The design handbook is considered in a way to support the whole development cycle of implementing a biochemical assay onto a centrifugal microfluidic system. It is used and maintained by the Lab-on-a-Chip Foundry Service of the HSG-IMIT and its backend, the Rapid-Prototyping

Service, to enable a fast realization of a biochemical assays in a miniaturized and integrated disposable microfluidic cartridge that can be automatically run by a stationary analyzer. Feedback from projects is directly added to the design handbook and labelled with project specific marks to protect our partners intellectual property rights.

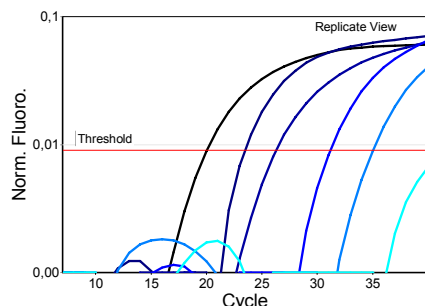


Fig. 6 Shown is the result of a DNA amplification experiment (increase of fluorescence with number of cycles) performed on a thermoformed foil in the RotorGene 2000® thermocycler.

The systematic development approach based on the Lab-on-a-Chip design handbook enables very fast development legs (Fig. 7). The aim of the Lab-on-a-Chip Foundry Service is to significantly reduce these legs down to twenty working days and less. Depending on the project phase this enables e.g. very fast feasibility studies.

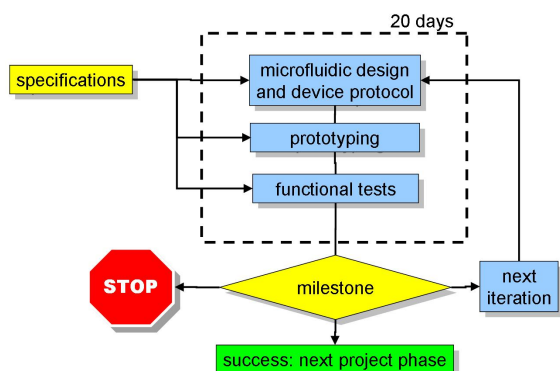


Fig. 7 Project flow with design iteration or project leg in 20 days enabled by the Lab-on-a-Chip Foundry Service.

### 3. Centrifugal microfluidic platform

Centrifugal liquid handling uses the interplay of centrifugal, capillary and pneumatic forces on a rotating microfluidic chip.

Liquid transport is initiated by the radial outwards directed centrifugal force  $f_{\omega}$  which can be scaled over a wide range by the frequency of rotation  $\omega$  together with the flow resistance of the fluidic channels. Small flow rates in the order of  $nL s^{-1}$  as well as high throughput continuous flows up to  $1 mL s^{-1}$  [7] can be generated. So scaling of flow rates over six 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.

Additionally the Coriolis force that works on liquid moving from the center to the periphery can be used for switching as it is dependent on the direction of rotation. The Euler force works on liquid samples during change of the frequency of rotation and can be used to induce convective forces for mixing of liquid samples.

The capillary force can be used to move liquid in the opposite direction than the centrifugal force or to prevent the movement of the liquid by hydrophobic barriers. Thus valves can be realized. Based on these forces, without any moveable parts on the centrifugal microfluidic platform a wide variety of microfluidic unit operations were developed by different research groups [8].

At the HSG-IMIT newly developed unit operations use the pneumatic response force of gas compartments in dead channels compressed by the centrifugal force on liquid plugs.

Thus, on the centrifugal microfluidic platform for the basic laboratory unit operations such as transport, valving, mixing, switching, metering, aliquotting a number of established solutions exist.

On the centrifugal microfluidic platform no expensive active valves are required and contact free dispensing avoids contamination of the disposable devices. We have already demonstrated colorimetric absorption assays for clinical chemistry (glucose, blood alcohol and hematocrite [9]), immunoassays [10], Real-Time PCR assays [11] and protein crystallization [12].

The centrifugal platform is a very powerful platform and products were already successfully developed elsewhere [4;13].

### 4. Rapid prototyping chains

The prototyping and fabrication of microfluidic systems for Lab-on-a-Chip applications is non trivial. A proper prototyping technology has to be as near as possible to the fabrication technology in mind for the later product development. For Lab-on-a-Chip applications the prototyping technology must not only allow a good fabrication of microstructures for the liquid handling, but must also be robust in a biochemical sense.

Typical problems that disturb the biochemical functionality of assays are unspecific adsorption of biofluids. Those fluids often contain a large amount of even not always completely known proteins, nucleic acids and other biopolymers. Because of the large surface to volume ratio in microfluidic systems, more molecule to wall interactions happen and the probability of disturbing effects such as unspecific bindings or denaturation of proteins happen.

While silicon and glass have advantages concerning the handling of biochemical liquids, polymers have to be used for a cost efficient fabrication of mass products for the diagnostics or environmental analysis markets. The typical mass fabrication methods for polymers like injection molding and thermoforming allow the cheap fabrication of high numbers of complete microfluidic cartridges.

Nevertheless, those fabrication routes come along with the demand of replication tools, whose fabrication for industry standards is rather expensive and makes the fabrication of such products economically reasonable only for an output above some hundred thousand pieces and more.

Microfluidic chips for experimental purposes can be fabricated rather simply by direct milling or hot embossing of plastics up to the numbers necessary for clinical or pre-clinical studies or customer side evaluations.

Nevertheless to provide a reliable fabrication, standardized prototyping chains are necessary and are set up at the HSG-IMIT Rapid-Prototyping Service. Such chains contain typical steps as shown in Fig. 8 and are compatible with mass fabrication technologies.

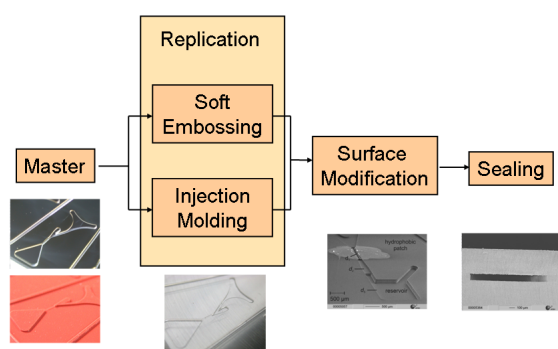


Fig. 8. Prototyping process flow in four steps.

First a master or replication tool of the cartridge is fabricated. Multiple chips are received by replication in polymer. Surface modifications and assembly steps are necessary depending on the biochemical assay. Finally the chip has to be sealed to enable the liquid control. While different technologies are available for the different steps, they have to be combined to standard fabrication chains that fit well to typical demands of the fabricated assay such as the miniaturization level.

For the fabrication of masters and tools, cleanroom processes based on silicon etching with chrome mask lithography are available for the smallest dimensions while micromilling is used for larger structures. Those processes together with standardized tooling facilities and dimensions enable the cost efficient application of replication processes in prototyping of microfluidic cartridges.

For the replication different technologies are available that can be used in prototyping and are compatible to mass fabrication. While soft embossing is a well established process for the fabrication of microfluidic systems, at the HSG-IMIT the thermoforming of foils for the fabrication of microfluidic structures has been implemented as well. A number of surface modifications for the blocking of unspecific bindings or the implementation of capillary driven unit operations are available including plasma deposition or wet coating e.g. with BSA.

An important task in the fabrication of microfluidic devices is the sealing of channels. The sealing must fluidically isolate different channels while not disturb the functionality of the cartridge, what can happen e.g. due

to the destruction of preloaded reagents during the sealing process or due to the chemical interaction of the process fluids with the sealing material or agents.

To quickly adapt processes and materials to different assays, at the HSG-IMIT compatibility tests for different assays get also standardized or are performed according to common standards with there local implementation documented in the design handbook. So e.g. the right sealing technology can be chosen without compromising the functionality of the assay.

## 5. Conclusions

Using the approach of microfluidic platforms enables the efficient and fast development of Lab-on-a-Chip Systems. 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 implementation of different application specific systems (assays) in an easy and flexible way, based on the same fabrication technology. For the successful application of a platform based development, a systematic workflow and rapid prototyping chains for the fast replication and assembly of microfluidic cartridges are necessary. At the HSG-IMIT such an integrated development environment has been set up and is offered to the community as the Lab-on-a-Chip Foundry Service. It provides a novel and highly integrated service for the realization of in vitro diagnostic assays as compact and automated diagnostic systems. It speeds up the development process by a systematic workflow with all necessary resources available in one hand.

## References

- [1] S. Haeberle and R. Zengerle; *Lab. Chip.*, 2007, 7, 1094 – 1110
- [2] J. Ducleé and R. Zengerle, *FlowMap - Microfluidics Roadmap for the Life Sc.*, 2004
- [3] Abbott Point-of-Care, USA
- [4] Abaxis Inc.
- [5] P. S. Dittrich and A. Manz, *Nature Reviews Drug Disc.*, vol. 5, no. 3, pp. 210-218, 2006
- [6] V. Hessel and H. Lowe, *Chem. Eng. & Tech.*, vol. 26, no. 1, pp. 13-24, Jan.2003.
- [7] S. Haeberle et. al., *Chem. Eng. & Tech.*, vol. 28, no. 5, pp. 613-616, May2005.
- [8] M. Madou et. al., *An. Rev. of Biomed. Eng.*, vol. 8, pp. 601-628, 2006.
- [9] J. Steigert et. al., *Lab Chip*, vol. 6, no. 8, pp. 1040-1044, 2006.
- [10] S. Lutz et. al., in *Proc. of the 11th Int. Conf. on Min. Sys. for Chem. and Life Sci. (μTAS) San Diego, US.*, 2008, pp. 1516-1518.
- [11] D. Mark et.al. in *Proc. of the 11th Int. Conf. on Min. Sys. for Chem. and Life Sci. (μTAS) San Diego, US.*, 2008, pp. 611-614.
- [12] C. P. Steinert et.al, in *Proc. of 19th Int. Conf. on Micro Elec. Mech. Sys. (MEMS) Kobe, Japan, 2007*, pp. 561-564.
- [13] Gyros AB, Sweden