2. Applications
(Bio-analytical systems)

Poster

THE LAB-ON-A-CHIP DESIGN & FOUNDRY SERVICE

S. Hin[1], D. Baumann[2], K. Mitsakakis[1,2], V. Klein[1], O. Strohmeier[1,2], M. Keller[1,2], D. Kosse[1,2], R. Zengerle[1,2,3], F. von Stetten[1,2], and D. Mark[1,2]

[1] Laboratory for MEMS Applications, Department of Microsystems Engineering - IMTEK, University of Freiburg, Georges-Koehler-Allee 103, 79110 Freiburg, Germany

[2] HSG-IMIT - Institut für Mikro- und Informationstechnik, Georges-Koehler-Allee 103, 79110 Freiburg, Germany

[3] BIOSS – Centre for Biological Signalling Studies, University of Freiburg, 79110 Freiburg, Germany

ABSTRACT

The Lab-on-a-Chip Design & Foundry Service offers the rapid development of point-of-care systems, which automate diagnostic assays delivered by the customer. The automation is done on a centrifugal microfluidic lab-on-a-chip platform, the LabDisk. The customer profits from the utilization of unit operations in the development process. Those unit operations apply standard rules for the design and production of the cartridges. Unit operations are combined to form process chains for the translation of a manual laboratory protocol into a fully automated analysis. Thus, risk, time, and costs are reduced during the development process, because a well-established production technology is used and existing developments can be re-used. To describe that development process, in this paper the example of a unit operation for liquid reagent release is implemented into a LabDisk for the EU-FP7 project DiscoGnosis. The liquid release is needed in a fluidic structure for nucleic acid extraction from pathogens out of a whole blood sample (50 µl). To implement this unit operation, five different buffer solutions are filled into stickpacks. For each buffer, a research-scale batch of 100 stickpacks is produced. It is demonstrated for all buffers that 4 out of 4 stickpacks release their content in the planned time in the protocol. The time effort for this first design iteration was two weeks, including specification phase, CAD, fabrication, and fluidic validation.

KEYWORDS

Lab-on-a-chip, foundry service, unit operations, centrifugal microfluidics, point-of-care diagnostics

INTRODUCTION

The development of lab-on-a-chip systems for diagnostic applications, such as the detection of infectious diseases, requires extensive knowledge in numerous fields of technology. Microfluidics, production technology, instrumentation, as well as biochemistry and medical knowledge interact with each other. They have to be well controlled and adjusted to achieve optimal performance of the system resulting in maximum benefit for the user. To minimize development effort for new applications, a "toolbox" that offers a set of unit operations is of advantage. Each unit operation represents a standard element for liquid handling to integrate a biochemical assay based on a well-established production technology [1]. By using this strategy we can offer centrifugal microfluidic systems developed with reduced risk, time, and costs.

Development strategy

Based on the customer's specifications, assays are automated on a centrifugal microfluidic platform, the LabDisk. This disposable foil based cartridge has the approximate foot-print of a CD (130 mm diameter) and is operated in a portable processing device, the LabDisk Player (Figure 1). A standard LabDisk Player comprises a motor to rotate the disc and to realize frequency protocols, magnets to handle magnetic particles inside the LabDisk, a heating and cooling unit for temperature control, and a detector for fluorescence and chemiluminescence.

Figure 1: The LabDisk, a foil based unit use cartridge processed by the LabDisk Player. (Source: HSG-IMIT/Bernd Müller)

Development process

At the beginning of a development process (Figure 2) the manual steps from the assay are translated into unit operations, e.g. reagent release, valving, mixing, and aliquoting. Connected to each other, a set of unit operations represents a process chain. A set of process chains is combined to implement a fully automated analysis.

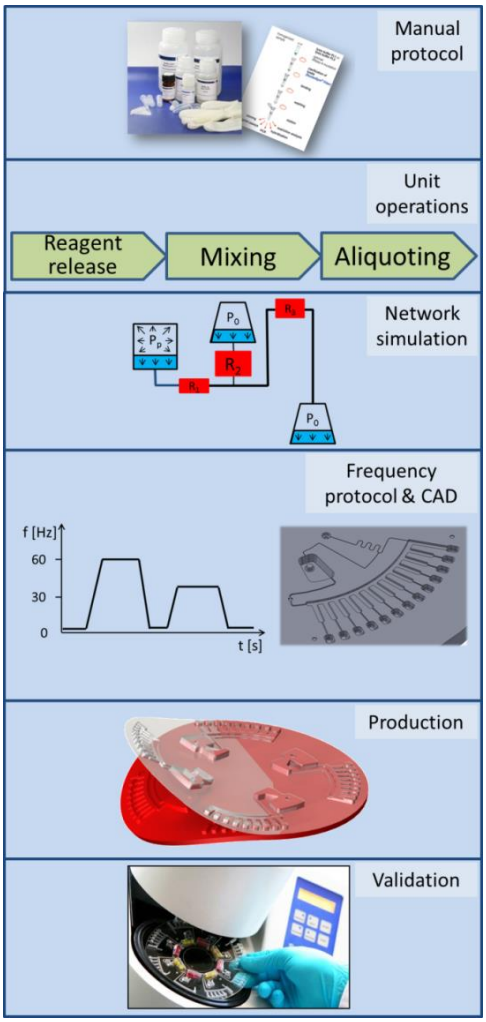

Figure 2: Principle of assay integration into a LabDisk. A manual protocol is translated into unit operations. Then, a network of unit operations is simulated to derive a frequency protocol and a CAD layout. Finally, the LabDisk is produced via micro-thermoforming, and subsequently validated.

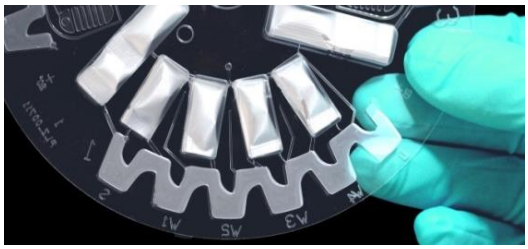

Figure 3: Stickpacks for liquid reagent pre-storage and release on demand inserted into a LabDisk.

The interfacing of the unit operations with each other is simulated using microfluidic network simulation, to derive a frequency and temperature protocol for the LabDisk Player and a CAD layout serving as a template for rapid prototyping of the microfluidic structures. Prototyping of the LabDisk is done via micro-thermoforming technology [2], which is adapted from blister production, a low-cost fabrication process used for drug or food packaging. Liquid reagents are pre-stored in stickpacks (Figure 3). These aluminum pouches allow long-term liquid storage and feature frangible seals, which are opened due to liquid pressure under centrifugation [3]. This enables a reagent release on demand at a desired rotational frequency. Dry reagent pre-storage is used for primers and probes in molecular testing, or magnetic beads in sample preparation. Prior to testing, the LabDisk is sealed with a pressure activated adhesive foil. The described production and packaging process enables small scale production of LabDisks (up to 100) for rapid prototyping, as well as pilot production batches for clinical validation (up to tens of thousands). We also offer to accompany our customers in the up-scaling process to mass production.

APPLICATION STUDY

Within the EU-FP7 project DiscoGnosis the Lab-on-a-Chip Design & Foundry Service develops the liquid handling environment [4]. The project DiscoGnosis aims to develop a multiplex diagnostic test to differentiate between tropical diseases causing fever, namely malaria, dengue, typho, and pneumonia. Detection of pathogen-specific nucleic acid targets via loop-mediated isothermal amplification (LAMP) should be performed in parallel with a bead-based heterogeneous immunoassay (Figure 4) on one LabDisk.

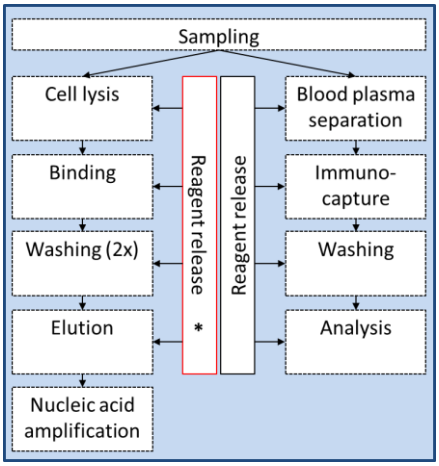

Figure 4: Process chains in DiscoGnosis project. The unit operation described in this paper is the reagent release for the process chains cell lysis, binding, washing, and elution (*).

By this approach, an enlarged diagnostic window is used, since both, nucleic acid targets and protein analytes are measured. All necessary steps are performed in a fully automated manner. This is of great advantage in resource poor settings with low training level of the user.

Task description

The unit operation, which should be implemented for the DiscoGnosis project, is a reagent release for buffers required in the nucleic acid extraction process (prior to LAMP). The reagent pre-storage and release is implemented for five different buffers and performed in parallel. A challenge is posed by the high number of necessary unit operations to perform two assays on one LabDisk. Therefore, a space-saving microfluidic layout is required to achieve the requested integration density. The presented layout solves this problem by placing the stickpacks radially inwards from the chambers to which the respective liquids are supplied (Figure 5).

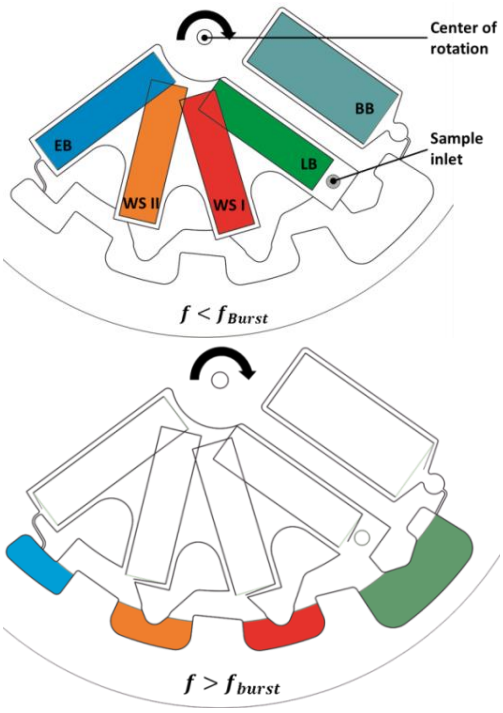

Figure 5: The unit operation of reagent release by stickpacks is shown. In the upper image, stickpacks are closed because the rotation frequency of the LabDisk is lower than the burst frequency. When the burst frequency is reached, stickpacks open and release the liquids to the nucleic acid extraction chambers. BB: Binding buffer; LB: Lysis Buffer; WS I: Washing solution I; WS II: Washing solution II; EB: Elution buffer.

Upon centrifugation of the LabDisk at a specified burst frequency, the stickpacks are opened due to hydrostatic pressure of the liquid acting on the frangible seal at the radially outer side. In this process, the burst frequency f_{Burst}, at which the stickpacks open, is given by

$$f_{burst} = \frac{1}{2\pi}\sqrt{\frac{2\,p_{liquid}}{\rho*(r_2^2-r_1^2)}} \qquad (1)$$

Where p_{liquid} is the required burst pressure, r_1 is the innermost radius, r_2 is the outermost radius, and ρ the liquid's density.

Specification

The specification process consists in here of three main steps (Table 1): First, the buffer volumes are specified in collaboration with the assay supplier (MagnaMedics Diagnostics B.V., Geleen/ the Netherlands). Then, the burst frequency, at which a stickpack releases its content, is defined by the foundry service based on a frequency protocol (data not shown) for the full process. Finally, the geometrical dimensions are defined in order to achieve a desired hydrostatic pressure according to equation (1).

Table 1: The specified parameters for stickpack production are shown.

Buffer solution	Volume* [µl]	Burst frequency** [Hz]
Lysis buffer	150	60
Binding buffer	440	75
Wash solution I	200	60
Wash solution II	200	60
Elution buffer	200	60

*: Parameter specified by customer/assay supplier. **: Parameter specified by fluidic protocol for the complete process.

Fabrication and Validation

The stickpacks are fabricated using a commercial three-side sealing-machine (Merz Verpackungs-maschinen, Lich, Germany). For fluidic validation, stickpacks are centrifuged on a laboratory centrifuge (Sigma1-15P, Sigma Centrifuges, Newtown, UK) at an outermost radial position of r_2=45 mm. On each stickpack, a frequency protocol of 1 min at 40 Hz and 1 min at 60 Hz is applied. After each single step, the integrity on the stickpack is visually checked. When 4/4 stickpacks of a certain type pass this protocol without opening at 40 Hz, but opening at 60 Hz, the test is passed. In this case, another two stickpacks are used for a final check, at which both have to open after 1 min at 60 Hz. This is necessary due to the preliminary applied load in the first test by the 40 Hz step. Thereby, a weakening of the sealing could occur, which would lead to a later falsification of results at the 60 Hz step.

RESULTS

Fabrication resulted in stickpacks with size of 9x33 mm² (washing solutions and elution buffer), 9x30 mm² (lysis buffer), and 15x32 mm² (binding buffer). The validation results are shown in Table 2.

Table 2: Validation results for stickpack burst frequency (opened stickpacks/tested stickpacks).

Buffer solution	Sequentially applied		Directly applied
	1 min 40 Hz	1 min 60 Hz	1 min 60 Hz
Lysis buffer	0/4	4/4	2/2
Binding buffer	0/4	4/4	2/2
Wash solution I	0/4	4/4	2/2
Wash solution II	0/4	4/4	2/2
Elution buffer	0/4	4/4	2/2

It can be seen that all stickpacks open at the desired burst frequency. As shown in Table 1, a burst frequency of 75 Hz is desired for the binding buffer stickpack. This can be achieved by placing that stickpack at a smaller radial position (e.g. r_2=39.5 mm). Then the hydrostatic pressure is comparable to the pressure during the validation experiment. From the data in Table 2 it cannot be concluded whether the stickpacks opened at 60 Hz or at some frequency between 40 Hz and 60 Hz. The time effort for the described task was two weeks. It has to be noted that the present results were obtained in the first iteration. For future projects that have comparable requirements, the development time will significantly reduce, due to the platform-based unit operation approach.

CONCLUSION

The Lab-on-a-Chip Design & Foundry Service offers the possibility to rapidly develop complex lab-on-a-chip systems like in the DiscoGnosis project. Based on the LabDisk platform, manual process steps are translated into unit operations that can also be re-used in further projects. Simulation, layout, production, and validation are offered from one supplier. This significantly minimizes development risk, time, and costs for our customers.

OUTLOOK

Biological validation can be performed in our biological safety level 2 laboratories. As we currently ramp up our production capacities to 50.000-200.000 LabDisks per year, the Lab-on-a-Chip Design & Foundry Service will be able to deliver sufficient numbers of cartridges for clinical validations.

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CONTACT

*S.Hin: sebastian.hin@imtek.de