Industry-supported, and standardized modular platform interconnecting fluidic circuit board and microfluidic building blocks

Stefan Dekker

Industry-supported, and standardized modular platform interconnecting fluidic circuit board and microfluidic building blocks

Stefan Dekker

Graduation committee

Chairman & Secretary:

Prof. dr. J.N. Kok

University of Twente

Promotor:

Prof. dr. ir. A. van den Berg

University of Twente

Co-promotor:

Dr. ir. M. Odijk

University of Twente

Referees:

Dr. ir. M. Blom

Micronit Microtechnologies

Dr. ir. N. Verplanck

CEA-Leti

Members:

Prof. dr. J.G.E. Gardeniers

University of Twente

Prof. dr. ir. B. Nauta

University of Twente

Prof. dr. P.J. French

Delft University of Technology

Prof. dr. R. Zengerle

University of Freiburg

The research described in this thesis was carried out at the BIOS Lab on a Chip group of the MESA+ institute for Nanotechnology and Techmed Centre for Biomedical Technology and Technical Medicine, University of Twente, Enschede, The Netherlands. The research was financially supported by the ECSEL Joint Undertaking under proposal number 621275-2, with the acronym MFManufacturing.

UNIVERSITY OF TWENTE.

Title: Industry-supported, and standardized modular platform
interconnecting fluidic circuit board and microfluidic building blocks

Author: Stefan Dekker

ISBN: 978-90-365-4611-9

DOI: 10.3990/1.9789036546119

URL: <https://doi.org/10.3990/1.9789036546119>

Cover design: Stefan Dekker

Publisher: Ipskamp Printing, Enschede, the Netherlands

Copyright © 2018 by Stefan Dekker, Enschede, The Netherlands.
All rights reserved.

INDUSTRY-SUPPORTED, AND STANDARDIZED MODULAR PLATFORM
INTERCONNECTING FLUIDIC CIRCUIT BOARD AND MICROFLUIDIC BUILDING BLOCKS

DISSERTATION

to obtain
the degree of doctor at the University of Twente,
on the authority of the rector magnificus,
prof. dr. T.T.M. Palstra
on account of the decision of the graduation committee,
to be publicly defended
on Friday 9 November 2018, 14:45

by

Stefan Dekker

born on 3 November 1989
in Assen, The Netherlands

This dissertation has been approved by:

Prof. dr. ir. A. van den Berg

Dr. ir. M. Odijk

Promotor

Co-promotor

Table of Content

Introduction.....	1
1.1 The microfluidic manufacturing consortium.....	2
1.2 Maturity of the microfluidic field	3
1.3 Thesis outline	5
1.4 References	5
The why and how of microfluidic standardization.....	7
2.1 Inhibiting factors for growth of the microfluidic field.....	8
2.2 How to overcome inhibiting factors and their influence on the standardization process...	12
2.3 Outlook.....	13
2.4 References	13
Standardized and modular microfluidic platform for fast Lab on Chip system development	15
3.1 Introduction	16
3.2 Standardization and design concepts	22
3.3 Standardization in physical dimensions	23
3.4 Methods.....	24
3.5 Test methods.....	28
3.6 Results and discussion	31
3.7 Characterization.....	33
3.8 Conclusions.....	35
3.9 Reference.....	35
Tools to design and simulate microfluidics	41
4.1 Introduction	42
4.2 Tools.....	43
4.3 Methods	46
4.4 Results and discussion	49
4.5 Conclusion	52
4.6 References	52
From chip-in-a-lab to lab-on-a-chip: a portable coulter counter using a modular platform.....	55
5.1 Introduction	56
5.2 Results & Discussion.....	58
5.3 Materials and Methods	61

5.4	Conclusion.....	65
5.5	References	66
Standardized PARallelization of multichamber Chips, the SPARC platform		69
6.1	Introduction	70
6.2	Design	71
6.3	Methods and materials	75
6.4	Results and discussion	79
6.5	Conclusion.....	85
6.6	References	85
Summary and outlook		87
7.1	Summary.....	88
7.2	Conclusion.....	90
7.3	Outlook.....	90
Appendix A - Design Guideline for Microfluidic Device and Component Interfaces (part 1)		93
A.1	Introduction	95
A.2	Definitions around chips and connectors	96
A.3	Pro forma standard chip sizes and interconnections.....	98
A.4	Standard guidelines for axes and reference point	100
A.5	Microfluidic port	104
A.6	Summary table of all interconnect guideline dimensions and tolerances regarding port pitches, chip thicknesses and port dimensions	109
A.7	Standard guide lines for chip formats	110
A.8	Exclusion zones	113
A.9	Sensor / Actuator building blocks	114
A.10	Standard guidelines for operational conditions / application classes	119
A.11	And Finally	121
Samenvatting		122
Scientific output.....		125
Funding and contributions.....		127
Dankwoord		129

Chapter 1

Introduction

In this chapter some background information is given about the overarching project in which this thesis was written. The necessity of the project is also discussed, by taking a look at the history of the microfluidic field and its current day status. A finishing section describes the structure of the rest of the thesis.

1.1 The microfluidic manufacturing consortium

The work described in the thesis laying before you was done under the umbrella of the Microfluidic Manufacturing project. The Microfluidic Manufacturing consortium was established to carry out the work proposed to the ECSEL Joint Undertaking, a public-private partnership funding agency focussed mainly in the area of electronic components and systems [1]. The overall goal of the project is to increase the maturity of the microfluidic field. To reach this goal the project focussed on two specific points. The first goal is the description and fabrication of standardized functional modules and their interoperability. The second goal is to make a distributed pilot line, making it possible to use fabrication processes at different partners for a single system. The work in this thesis focuses mainly on the first point. To realize standardized parts, usable for the whole field of microfluidics a wide range of partners was involved. These partners include large research institutes, large multi-nationals, and several smaller start-up companies. A list with the involved partners from many of the branches in the microfluidic field can be found below. The project was funded by the ECSEL JU agency under project number 621275-2 and coordinated by Marko Blom from Micronit. The project is better known under the acronym MFManufacturing.

Academic and Research institutes:

- University of Twente
- Nederlandse Organisatie voor toegepast-natuurwetenschappelijk onderzoek (TNO)
- Commissariat à l'énergie atomique et aux énergies alternatives (CEA)
- National Physical Laboratory management Limited

Microfluidics manufactures:

- Micronit
- Dolomite
- Axxicon
- STI plastics
- Tronics microsystems

Product/Demonstrator partners:

- Philips
- Medimetrics
- APIX
- EVEON
- PMB

Software development:

- Viseo
- Phoenix

Equipment manufacturers:

- Fluigent
- Dolomite

Management partners:

- enablingMNT

Unofficial support:

- Microfluidic Chipshop

1.2 Maturity of the microfluidic field

It is hard to say when the microfluidic field exactly started. The paper published by Andreas Manz, Norbert Graber and Michael Widmer in 1990 [2] is often seen as the starting point of the microfluidic field. In this paper, the term micro total analysis system (μ TAS) is introduced. At the time, microfluidics started as a side branch of the micro-electromechanical system (MEMS) field. Typical advantages of this new technology are the use of low sample and reagent volumes, faster processing time due their small scale and therefore low diffusion time scales and the resulting portability of systems. The microfluidic field grew rapidly starting as a side branch of the MEMS field to a field standing on its own, having its first own μ TAS conference in 1994 [3]. From the start the microfluidic field has shown a rapid growth. The increase in published papers and patents can be seen in fig. 1.1. To analyse the maturity of the field the so-called hype cycle can be applied. It was developed by Jackie Fenn while working for the research and advisory firm Gartner [4]. The hype cycle consists of five consecutive phases: technology trigger, peak of inflated expectations, trough of disillusionment, slope of enlightenment, and plateau of productivity. In the technology trigger phase, a technology breaks through and generates a lot of publicity. This is followed by the emergence of first products which are impractical and very expensive, however media attention is still very high. The next phase is initialized by unmet expectations, resulting in loss of attention from media and the general public. During the slope of enlightenment the industry continues to improve the technology and finds applications for its capacities. Finally, the plateau of productivity is reached where the technology becomes mainstream.

Figure 1.1 - Number of published papers and patents from 1988 to 2013. Reproduced from Ref. [5] with permission from The Royal Society of Chemistry.

Looking at fig. 1.1 the technology trigger phase occurred around 1990, as the μ TAS concept was introduced by A. Manz [2]. Expectations were high at the time as microfluidics was a side branch of microelectronics field with successes such as Moore's law and a bit later the starting sales of MEMS accelerometers for the use in the automotive industry. From the nineties on expectations were expanded until about 2003 when the peak of inflated expectations was reached. The promised miniaturization of for example the medical diagnostics for the developing world were for example not yet realized[6]. After this, the amount of journal articles published keeps increasing, but the amount published patents stabilizes. However, the public is not interested anymore and media attention declines. The hype is over and the field is landed in the trough of disillusionment. One could argue that 10 years after the start of the field, no killer application, like the processor for the microelectronics field or the accelerometer for the MEMS field, was found [7]. However, a sizable market has developed around DNA sequencing. Actively involved people kept developing the technology for other niche markets moving along the slope of enlightenment, slowly building a market for itself, as an enabling tool for specific custom applications. The strength of the microfluidic field is that these applications can be very broad e.g. chemical analysis for food or water testing, point-of-care diagnostics, bio-threat detection or as chemical reactors [8]. The field is therefore moving toward a stable plateau for productivity based on a business-to-business market.

1.3 Thesis outline

To provide justification for the work presented in this thesis, chapter 2 describes factors that inhibit the growth of the microfluidic field and explains how standardization could take away some of these inhibitions. Furthermore, the impact of the inhibiting factors on the standardization process itself is discussed. chapter 3, shows the results of the standardization process and gives a toolbox to design standardized modular systems. Building further on this, chapter 4 combines the standardized functional blocks with a computer aided design tool and shows some initial simulation capabilities. After these more general chapters, there are two application specific chapters, showing the implementation of the standardized modular functional elements. Chapter 5 describes a coulter counter system in a compact form factor capable of counting micrometre sized beads. Chapter 6 describes a high-throughput screening system with 192 individually addressable chambers. Finally, a concluding summary is given with an outlook on the future of the standardization effort.

1.4 References

- [1] Shaping digital innovation | ECSEL Joint Undertaking, (n.d.). <https://www.ecsel.eu/> (accessed July 5, 2018).
- [2] A. Manz, N. Graber, H.M. Widmer, Miniaturized total chemical analysis systems: A novel concept for chemical sensing, *Sensors Actuators B Chem.* 1 (1990) 244–248. doi:10.1016/0925-4005(90)80209-I.
- [3] P. Bergveld, The Challenge of Developing μ TAS, in: *Micro Total Anal. Syst.*, Springer Netherlands, Dordrecht, 1995: pp. 1–4. doi:10.1007/978-94-011-0161-5_1.
- [4] J. Fenn, M. Raskino, *Mastering the hype cycle : how to choose the right innovation at the right time*, Harvard Business Press, 2008.
- [5] A.K. Yetisen, L.R. Volpatti, Patent protection and licensing in microfluidics, *Lab Chip.* 14 (2014) 2217. doi:10.1039/c4lc00399c.
- [6] X. Mao, T.J. Huang, Microfluidic diagnostics for the developing world, (n.d.). doi:10.1039/c2lc90022j.
- [7] G.M. Whitesides, The origins and the future of microfluidics, *Nature.* 442 (2006) 368–373. doi:10.1038/nature05058.

[8] H. Becker, Hype, hope and hubris: the quest for the killer application in microfluidics, *Lab Chip*. 9 (2009) 2119. doi:10.1039/b911553f.

Chapter 2

The why and how of microfluidic standardization

This chapter describes the most important difficulties encountered in developing a microfluidic device and subsequently commercializing it. It is followed by a discussion on how to overcome some of these difficulties by standardization. The influence of these factor on the standardization process itself is also discussed.

2.1 Inhibiting factors for growth of the microfluidic field

2.1.1 Multidisciplinary

8 Microfluidic devices are often complex, requiring a multidisciplinary approach due to the various physical and chemistry domains involved. These domains are physics, material science, chemistry, biology, electrical engineering and mechanical engineering. They support the design of a microfluidic device in the following respective areas: predicting the behaviour of small amount of fluids, fluid surface interactions, sample and reagent use, cell culturing, sensors and their read-out and integration into a product [1]. With the expertise of all these professionals very complex microfluidic devices can be realized, but this requires effective communication between all these disciplines. With each discipline having their own background with corresponding vocabulary, this effective communication turns out to be difficult in practice. An example with the word dye and die, which are both pronounced as /daɪ/, is given below. Depending on the background and expertise, these words can mean very different things. The layman thinks somebody is death or something has ceased to exist. The electrical engineer is talking about a piece of silicon containing the electrical functionality of an integrated circuit, a wafer often holds multiple dies. The mechanical engineer thinks about injection moulding, as a mould for these machines is also called a die. For the chemist it has a different meaning again, they talk about a colouring or labelling compound. The problem is that in the microfluidic field they are all in context. Think for instance about the following sentence: “The pocket in the die has to match the size of the sensor die, so that the dye flows over the sensor die.” It doesn’t make much sense, but could be used in a microfluidic context. A translation: The pocket in the mould has to match the size of the sensor integrated circuit, so that the coloured solution flows over the silicon sensor area.”

Besides the communication, a multidisciplinary team consists of several professionals with corresponding costs, as discussed in the next section.

2.1.2 Economy of scale

As already stated the development of a microfluidic device often requires a multidisciplinary team, meaning high cost even before one can think about production. When arriving at a production-ready state, additional costs occur for production equipment, design specific masks or moulds and raw materials. To obtain the required functionality of the device, often back-end processing like surface functionalization is needed [2]. Holger Becker used equation 2.1 to describe the cost of a single microfluidic device [2].

$$C_{dev}(n) = C_{mat} + \frac{C_{NRE}}{n} + C_0 e^{-(\log n/N)} \quad (2.1)$$

9

Where C_{dev} is the cost per device, originating from C_{mat} the material cost, C_{NRE} are the non-recurring costs like design hours and device specific moulds or masks. At last C_0 , the initial process costs with N being the process-specific cost scaling factor. The equation shows that with increasing production number the cost per device goes down. In a previous section the multidisciplinary design team and corresponding high development costs were discussed, resulting in the need of high production volumes to reach acceptable costs per device. However, the current microfluidic market focusses on specific custom applications, which are generally of smaller volume. This results in a standstill: small volumes are possible, but it reduces the amount of possibilities as the non-recurring costs need to be kept low.

2.1.3 Order of magnitude difference in volumes

Another consequence of the niche microfluidic market is the broad range of the fluid volumes and flow rates involved. For example, the on-site preparation/re-suspension of drug requires the production of millilitres of liquid [3], while on the opposite side nanopore biosensors confine the analytes to a volume of only a few zeptolitre (10^{-21} L) [4]. So between these two extreme examples there is a volume difference of 18 orders of magnitude.

2.1.4 Intellectual property and patents

Any research or development work results in some degree of intellectual property. One of the ways to protect this intellectual property is the filing of a patent. A patent gives the inventor the sole right to use the invention for a fixed period of time (often twenty years). In return for this right, the inventor has to describe how the inventions functions, which becomes public knowledge after the filing

of the patent. The system of patents was introduced by Queen Elizabeth I (1558-1603) issuing royal grants for exclusive privileges. In the beginning of the nineteenth century, a patent provided the inventor the legal right to control the production and sale of his invention [5], which is close to its current form. A few requirements for a patent to be granted are: (1) It has to work, (2) It has to be novel (Inventions known to the public domain will not get a patent granted), (3) It has to be non-obvious and (4) It has to be functional or technical, how something works or how something is made [6].

In the economy of scale section, we discussed that the non-recurring cost for a microfluidic device will be substantial, mostly because of the research and development work. It is natural to safeguard this research and development work by applying for a patent. Figure 2.1 shows that over the years, many inventions were patented in the microfluidic field. Taking a 20 year lifespan into account [7], means that at the moment of writing around 40 000 patents are active in the microfluidic field. This is a good sign, showing that the field is active and making new inventions. However, for start-up companies this amount of patents is daunting, as it is almost impossible to check whether your new technology infringes on already existing patents.

Figure 2.1 - Patents granted since 1990 with the word microfluidics mentioned in them [8]. The total amount of patents granted in this time frame is 42163.

2.1.5 Materials and their fabrication methods

Nowadays, the variety of materials used in the microfluidic field is large. However, in the beginning mostly glass and silicon substrates were used to fabricate microfluidic devices. For these materials, micromachining technologies were already available from the MEMS field. The field evolved over the years and more and more materials caught the interest of microfluidic researchers. Currently, the most used materials in the field are glass, silicon, polymers and paper [9]. The polymer category is broad and contains many materials, but we will focus on two in the subcategories elastomers and thermoplastics. The elastomer Polydimethylsiloxane (PDMS) is one of the most favourite materials of the microfluidic research community, as it has a reasonable cost and can be used for rapid prototyping. Moreover, due to its flexibility it is also possible to integrate active components like valves into the design. However, for production purposes, PDMS casting is not ideal, as the recurring costs are relatively high [10]. The transition to a commercially more suited material like the Food and Drug Administration (FDA) approved thermoplast cyclic-olefin-copolymer (COC), is difficult. A main challenge comes from the fact that for COC a different high volume manufacturing process such as injection moulding or hot-embossing has to be used. The change of material often undoes the previous design iterations. The need for investments in both the design and manufacturing equipment make it difficult to step from a PDMS demonstrator in the lab to a production-ready prototype in a different material.

2.1.6 Do it yourself

Especially in a research environment it is common to have a PhD student do the whole process from design, material choice to the actual fabrication. This means lots of wheels are being reinvented. In an academic setting this is defensible, as students need training, so a hands on approach is beneficial. However, if for example the fabrication is done externally, more time is available to test the functionality of the device and maybe even some steps towards commercialization can be done. However, this requires a change in the academic system/mentality where currently a publication is the most important achievement.

2.1.7 Academy vs industry

In academic work a proof of concept system that can be published is often the end point of the work: it was proven that the system works. Maybe only once, but enough to get published. To then make the transition to a marketable product, where every device has to work, is difficult. In the current market it is

important for the industry to quickly get a return on their investment, meaning there is no time and money for research and development, which is necessary to bring proof of concepts to the level of a marketable product [1].

2.2 How to overcome inhibiting factors and their influence on the standardization process

12

From the beginning of the MFManufacturing project it was clear that the solution should be a modular platform that interconnects several microfluidic parts. The proposed modularity can tackle some of the inhibiting factors described in the previous section. Packing the multidisciplinary design effort into a functional module, provides the possibility to reuse this module. This has a twofold advantage: the modules can be produced in higher numbers, making them cheaper and the next design can benefit from already existing modules, preventing the need for reinvention of the wheel. Over time, a library with modules will be available. Another advantage is the possibility to work both top down and bottom up. For a bottom up approach, the manufacturing method is leading in structures created on the chip and eventually what functionally is created. The top down approach starts with a functional description of the system, after which it is broken down into smaller parts which are then realized. On a modular platform the system can be designed in a top down manner until the level of the modules is reached. The modules themselves can be designed bottom up. An advantage hereof is that expertise can be compartmentalized, reducing the multidisciplinary inhibitor.

However, this approach requires the individual modules to be compatible and interconnectable, this is where standardization comes in. The next question is: “What do we standardize?”. Fortunately, all partners in the project saw potential in standardization and the potential growth of the microfluidic field. They were willing to share and collaborate with their direct competitors. With the idea that, as a result, they might only get a smaller part of a bigger pie, but in the end still have more pie. However, in the following discussion the IP and patents inhibitor as well as the multidisciplinary inhibitor popped up. Involved parties did not want to harm the position of their company and had to be careful with what IP to share. The multidisciplinary inhibitor caused lots of jumps from detailed discussion back to general discussions, slowing down progress within the project. Finally, a consensus was reached: only the external dimensional requirements were standardized. This left the internal implementation to the individual partners in the project, so they were able to put their IP inside a standardized module. System components and corresponding names were set as follows: a

microfluidic building block (MFBB) is a modular building block containing a certain microfluidic function. Several of such blocks can be connected together through a fluidic circuit board (FCB) which provides the connection between the MFBBs. These connections can be of fluidic, electric or, in the future, optic nature. Requirements were set for outside dimensions, locations of the inlets and outlets and zones were defined that should be kept empty. Besides mechanical standardization, also application classes were defined based on maximum allowable temperature and pressure. MFBBs and FCBs can be classified as being compatible with certain application classes. Results of this standardization process are published in two design guidelines [11,12] and the most important parts are formalized in an ISO International work shop agreement [13].

2.3 Outlook

Throughout the project it was promising to see that the involved parties saw the value and benefits of standardization in the microfluidic field. This standardization makes it possible to use a modular platform to develop microfluidic devices, which in turn provides a stepping stone towards commercial microfluidic products. However, for this standardized modular platform to take off, it is important that microfluidic systems can be design in a top down manner, thereby relieving some of the multidisciplinary needs. A library filled with functional building blocks is needed to achieve this goals. In chapter 3 of this thesis, a start of such a library is given with input and contributions of several companies and academic institutes. Over time this libraries needs to be expanded to fully benefit from the standardized modular platform. At present, the standardization already provides a common way to connect auxiliary components to microfluidic chips.

2.4 References

- [1] H. Becker, Lost in translation, *Lab Chip*. 10 (2010) 813. doi:10.1039/c002744h.
- [2] H. Becker, It's the economy..., *Lab Chip*. (2009). doi:10.1039/b916505n.
- [3] Intuity® Mix Platform, (n.d.). <http://eveon.eu/en/solutions-and-services/intuity-mix-platform> (accessed June 26, 2018).
- [4] T. Review, B.N. Miles, A.P. Ivanov, K.A. Wilson, F. Doğan, D. Japrun, J.B. Edel, *Chemical Society Reviews* Single molecule sensing with solid-state

nanopores: novel materials, methods, and applications, *Chem. Soc. Rev. Chem. Soc. Rev.* 42 (2013) 306–12. <http://pubs.rsc.org/en/content/articlepdf/2013/CS/C2CS35286A> (accessed June 26, 2018).

[5] A. Mossoff, Rethinking the Development of Patents: An Intellectual History, 1550-1800, *Hast. L.J.* 52 (2000) 1255–1322. http://heinonlinebackup.com/hol-cgi-bin/get_pdf.cgi?handle=hein.journals/hastlj52§ion=49 (accessed June 27, 2018).

[6] H. Becker, IP or no IP: That is the question, *Lab Chip.* (2009). doi:10.1039/b920548a.

[7] A.K. Yetisen, L.R. Volpatti, Patent protection and licensing in microfluidics, *Lab Chip.* 14 (2014) 2217. doi:10.1039/c4lc00399c.

[8] Depatisnet, (n.d.). <https://depatisnet.dpma.de/DepatisNet/> (accessed June 27, 2018).

[9] P.N. Nge, C.I. Rogers, A.T. Woolley, Advances in Microfluidic Materials, Functions, Integration, and Applications, *Chem. Rev.* 113 (2013) 2550–2583. doi:10.1021/cr300337x.

[10] R. Mukhopadhyay, When PDMS isn't the best, *Anal. Chem.* 79 (2007) 3248–3253. doi:10.1021/ac071903e.

[11] H. van Heeren, T. Atkins, N. Verplanck, C. Peponnet, P. Hewkin, M. Blom, W. Buesink, J.-E. Bullema, S. Dekker, Design Guideline for Microfluidic Device and Component Interfaces (part 1) ver. 2, 2016. doi:10.13140/RG.2.1.1698.5206.

[12] H. van Heeren, D. Verhoeven, T. Atkins, A. Tzannis, H. Becker, W. Buesink, P. Chen, Design Guideline for Microfluidic Device and Component Interfaces (Part 2) ver. 1.2, 2016. doi:10.13140/RG.2.1.3318.9364.

[13] IWA 23:2016 - Interoperability of microfluidic devices -- Guidelines for pitch spacing dimensions and initial device classification, n.d. http://www.iso.org/iso/home/store/catalogue_tc/catalogue_detail.htm?csnumber=70603.

Chapter 3

Standardized and modular microfluidic platform for fast Lab on Chip system development

Since the Lab on a Chip concept was introduced in the 1990s, a lot of scientific advancements have occurred. However, large scale commercial realization of microfluidic technology is being prevented by the lack of standardization. There seems to be a gap between Lab on a Chip systems developed in the lab and those that are manufacturable on a large scale in a fab. In this chapter, we propose a modular platform which makes use of standardized parts. Using this platform, a functional-based method of designing microfluidic systems is envisioned. To obtain a certain microfluidic function, a bottom-up design is made. This results in micro fluidic building blocks that perform a microfluidic function. This microfluidic building block is then stored in a library, ready for reuse in the future. Key characteristics are shown for several basic microfluidic building blocks, developed according to a footprint and interconnect standard by various players in the microfluidic world. Such a library of reusable and interoperable microfluidic building blocks is important to fill the gap between lab and fab, as it reduces the time-to-market by lowering prototype time cycles. The wide support of key European players active in microfluidics, which is shown by an ISO workshop agreement (IWA 23:2016), makes this approach more likely to succeed compared to earlier attempts in modular microfluidics.

This chapter is as published: S. Dekker, W. Buesink, M. Blom, M. Alessio, N. Verplanck, M. Hihoud, C. Dehan, W. César, A. Le Nel, A. van den Berg, M. Odijk, Standardized and modular microfluidic platform for fast Lab on Chip system development, *Sensors Actuators B Chem.* 272 (2018), 468–478.

3.1 Introduction

The concept of Lab on a Chip (LOC) and micro total analysis (μ TAS) systems was introduced in 1990 by Manz et al., one of the pioneers in the field [1]. Seventeen years later, the field has already showed major advancement by demonstrating many promising concepts for the various components such as sample prep, separation and detection that are combined into a μ TAS. Yet in 2006 Whitesides argued that the field had not yet fully reached its potential, discussing the typical struggles faced by new technologies including the ease of use for non-experts, and the transfer of technology from academy to industry [2]. Today, microfluidic technology still has not fully become mainstream technology. It appears that there are still many hurdles to overcome when migrating an idea from academics into a product ready for the market [3].

16

To bridge the gap between academic research efforts and the utilization of microfluidic technologies to address real world problems, standardization is essential [4]. Often, monolithic, by which I mean out of one part, Lab on Chips are developed, integrating several functions onto a single device. This approach often leads to the repeated development of already existing concepts, resulting in long development times. The need for high investments makes it exclusively economic for large volumes. Instead, a modular approach could significantly speed up development and prevent the waste of development resources by not “reinventing the wheel”. Less development effort is needed in modular systems as standardized parts of the system can be reused. The electronics industry can be taken as a good example of where such standardization works well. In that industry, standards exist for almost every aspect from package dimensions, to standard classes for printed circuit board manufacturing, to solder joints. The development of standards moved the electronics industry from the early “spider web assembly” in the 1950s to the complex system-on-a-chip technology of today.

To show that standardization doesn't exist, at least up to the level of interoperability, in the state of the art modular microfluidics, a list is made in Table 3.1. Several papers and industrial efforts to produce modular microfluidic systems are shown. It can be seen that although the systems are modular, they are definitely not standardized and thereby preventing interoperability between various modular systems. The table shows elements needed to obtain a functional system, from interconnects to functional blocks. In our approach standardized footprints and standardized interconnect grids are used, we see a future with interoperable modular blocks to build microfluidic systems. Looking towards

the future and bigger production volumes, several examples are included where modularity is used during the design phase; linking functions together, but still producing a monolithic device.

Table 3.1 - Overview of modularity in microfluidics

Inventor(s)	Type of fluidic part	Main material	Total system	Typical application field	Modular in Physical part/design	Reversible
Academics						
Lammerink et al. [5]	Functional blocks connected by a base board.	Si/Glass	Yes	Chemistry	Modularity in physical sense	No, modules are permanently fixed to the base board
Gonzalez et al. [6]	Interconnects for assembly of a modular system	Si/Glass	No, only focused on interconnect	Broad application	Modularity in physical sense	Yes, Silicone O-ring are used so the connection is reversible.
Gray et al. [7]	Interconnects for assembly of a modular system	SI/Glass device Plastic Coupler	No, only focused on world to chip interconnects	Broad application	Modularity in physical sense	Yes, but a new coupler might be required
Schabmuller et al. [8]	Functional blocks connected by a base board.	Si/Glass	Yes	Chemistry	Modularity in physical sense	No, modules are anodically bonded to the base plate.
Wego [9]	Functional blocks made in PCB technology	Copper plated FR-4	No, a few component are shown in PCB technology	Broad application	Modularity in physical sense	Yes, tubing is used for interconnection.
Rhee [10]	Functional block connected directly to each other	PDMS	Yes	Biological, PCR and cell culturing	Modularity in physical sense	No, Adhesive is used to interconnect the blocks
Strohmeier et al. [11]	Unit operations connected together in a monolithic centrifugal device	Mainly polymer	Yes	Broad application	Modularity in design	No, a monolithic device is fabricated
Yuen [12–14]	System build entirely out of blocks	Polymer with 3d printing as structuring method	Yes	Simple systems	Modularity in physical sense	Yes, a mini Luer or magnet is used to connect the blocks.

Vittayaruk-skul et al. [15]	System build entirely out of blocks	PDMS	Yes	Simple systems	Modularity in physical sense	Yes, compression of PDMS is used to make a fluidic seal.
Shaikh et al. [16]	Interconnects are made on a base plate containing multiple functionalities.	PDMS Silicon	Yes	Biochemical analysis	Modularity in design	Not easy, as the PDMS is bonded to the Silicon.
Millet et al. [17]	Functional units are connected by 3d tubes	PDMS	Yes	Biochemical analysis	Modularity in design	No, a monolithic PDMS device is casted.
Bhargava et al. [18]	System build entirely out of blocks	Polymer with 3d printing as structuring method	Yes	Droplet based applications	Modularity in physical sense	Yes, an elastic reversible seal is used
Loskill et al. [19]	Different organ chambers, interconnectable with connectors	PDMS	Yes	Organ-on-a-chip	Modularity in physical sense	Yes, connections made with the connector blocks are reversible
Industry						
Lionix [20]	Functional blocks are mounted in a PCB/base board	FR-4 Si/Glass	Yes	Chemistry	Modularity in physical sense	Yes, modules are mechanically fixed by soldering and sealing is done with O-rings.
Epigem [21]	Functional blocks connected by a base board	Polymer based	Yes	Broad application	Modularity in physical sense	Yes, modules are mechanically held down. A PTFE ferrule provides the seal
Labsmith [22]	Functional blocks mounted on a base board, connected by tubing	Several	Yes	Broad application	Modularity in physical sense	Yes, Tubing connectors are reversible

One of the earliest modular concepts was developed by Lammerink et al., introducing the concept of a Mixed Circuit Board (MCB) [5]. The board consisted of a printed circuit board and a polycarbonate substrate, respectively responsible for the electrical and fluidic interconnects between actuator and sensor modules. Others focused more on the interconnect itself. Gonzalez et al. [6] described a self-aligning reversible interconnect. Grey et al. [7] used a different approach using plastic press fit couplers to connect tubing to a silicon system. A system similar to the mixed circuit board, but using anodic bonding instead of adhesives, to mount the functional parts on the interconnect parts was developed by Schabmuller et al. [8].

Initial efforts to produce a modular microfluidic system often used silicon or glass, well known from MEMS technology. However, a disadvantage of these materials is that they are only economically feasible if large numbers are produced. For the functional modules this is not a problem, as these can be manufactured in high numbers. However, the interconnect solution is often application-specific and thus tends to be produced in lower numbers.

Wego et al. [9] looked at printed circuit board technology to fabricate integrated microsystems. Printed circuit board technology has less accurate dimensional tolerances than conventional fabrication methods used in the microfluidic field. However it is cheaper, especially when producing low number volumes. By the introduction of polymer layers in the stack, they were able to perform microfluidic functions.

Other materials were also investigated for use in modular systems. Microfluidic assembly blocks, (MABs) made from PDMS were introduced by Rhee et al. [10]. They are mounted side by side and sealed by an adhesive.

Lego© was an inspiration for Vittayarukskul et al. [15] who produced a fully reversible microfluidic system based on PDMS Lego blocks. The elasticity of PDMS was used to provide a seal between the blocks. Another plug-and-play system was developed by Yuen [12]. Stereo lithography 3D printing was used to fabricate the blocks for this system, which were interconnected using mini-Luer connections. Miserendino [23] showed a system used a clamping to seal, with patternable silicone micro gaskets between the baseplate and functional blocks.

Strohmeier et al [11] used a different approach when they defined a functional unit cell. With these functional unit cells they designed centrifugal microfluidics

devices. Using modularity in the design phase while still producing a monolithic device. Millet et al. [17] achieved something similar, but then for PDMS devices. On the pouring mould they added Acrylonitrile butadiene styrene (ABS) strings between the various components to create integrated tubing in the casting.

The microfluidic industry itself has also looked for solutions to interconnect microfluidic systems. One example of such a solution is the MATAS platform [20,24]. This platform is based on PCB technology with the addition of an extra layer for the fluidics. Blocks implementing microfluidic functions are placed inside milled cavities in the PCB and are connected to the fluidic layer by using O-rings. The blocks are fixed in place by solder. Another platform was developed by Epigem [21] that is similar to the MATAS platform, but instead of PCB technology it is fully based on thermoplastics. Labsmith opted for a slightly different system in which the modules are mounted on a board and for interconnections tubing is used.

From the above it is clear a large variety of modular platforms exists, both in terms of the level of integration in a single module and the place where the modularity is implemented. Hereby, making reuse of the modules of several platforms difficult.

In the future we foresee modularity in both; physical blocks in the end product and already during the design process. At one end of the spectrum is the unit cell operation approach during the design phase used by Strohmeier et al [11] and the plug-and-play systems of Yuen et al [12–14] at the other. However, it would be beneficial if these approaches could be used in conjunction with each other; for example if the auxiliary components of the system are in a plug-and-play fashion while the main chip can be designed using functional units. In this chapter we focus on the plug-and-play system for auxiliary components. To reach this, some degree of standardization is needed. Unfortunately, development of these modular platforms until now is done mostly independently by small groups of interested parties. The modular platform proposed in this chapter strongly argues for standardization. A large multinational consortium is backing and co-developing this standard [25]. The focus lies on the ability to interconnect parts from various suppliers together. With this we hope to attain a flourishing ecosystem in which microfluidic parts produced using various techniques (polymer, glass, and silicon) are both available and interconnectable. To help the end user, a library of standardized parts and functionality is also developed, supported by software managing the complete pipeline from design to

the production of a microfluidic system [26]. If we draw another analogy from the electronics industry, this library could be regarded as the catalogue of big component suppliers such as Newark and Farnell. Using schematics and routing software, these components together are designed to function as complex electronics devices.

Following our approach, we make use of a combination of microfluidic building blocks (MFBB) and fluidic circuit boards (FCBs). In this approach, the MFBB contains the fluidic functionality and the FCB connects all the building block together in a microfluidic system. Both the MFBBs and FCBs are designed and fabricated by industrial and academic partners according to guidelines, documented in a ISO workshop agreement [27–29]. This standardized approach makes it possible to reuse modules and have them interoperable between several partners. Moreover, it allows for a top-down design approach saving valuable development time. Having industrial partners inside the project gives the prospect of having commercial of the-shelf-parts available in the future.

3.2 Standardization and design concepts

3.2.1 Define specification from requirements

When designing a microfluidic system it is, of course, important to know what the requirements for the individual system are. Moving forward, decisions are made with regard to the specifications of the microfluidic system. From this point, a start is made with the physical realization of the system. In the microfluidic world, a bottom up approach is often used where the fabrication technology plays a large role in the design considerations. An important advantage of using a modular platform is that the functional design and physical design can be decoupled. Again, using the analogy with the electronics industry, this would translate to the functional design described by a schematic, while the physical design is the layout of all the transistors inside an integrated circuit. Accordingly, a top down design scheme can be used for the development of the microfluidic system.

Functional vs. physical design

This decoupling of the functional design and physical design makes it possible to work with standardized functional blocks, which only need to be designed and created once. These standardized functional blocks give a microfluidic designer the opportunity to focus merely on the function of a microfluidic system. For

this system of standardized microfluidic blocks, most of the common functions should be available to the designer in a standard form. The designer should also have the opportunity to design new blocks that have a specific functionality, but which still conforms to the standard. A designer can be assisted by making use of a CAD system. A library containing the functional building blocks helps the designer to quickly create a new microfluidic system and prevents the reinvention of the wheel often seen in microfluidics.

3.2.2 Flexibility

The flexibility in this system is the freedom to choose how to interconnect the building blocks together. This flexibility finds its implementation in the FCB. This means that each system has its own custom implementation of a FCB. Nevertheless, the interface between the FCB and the MFBB remains standardized. This provides practical advantages such as the second sourcing of parts from various suppliers and the ability to interchange building blocks that have slightly varying functionality. Most of the interfacing hardware is situated in the FCB, so the interfacing can be made to fit the requirements that are specific to a particular application.

3.3 Standardization in physical dimensions

To make this building block and FCB combination work, interoperability between the various components is needed. Therefore, there is a need to standardize the outside dimensions. This makes it possible to use a standardized system to connect the building blocks to the FCB. To align the ports, a standard grid is used as shown in figure 3.1. Inlet and outlets are placed on this grid. Furthermore, the standard dictates that the sealing between the FCB and MFBB is realized in the FCB, which seals to the flat bottom of the MFBB. How this seal is realized is up to the manufacturer of the FCB, providing a possibility for industrial partners to distinguish themselves. An example with O-rings is shown in figure 3.1E.

Within the standard framework, there are several options (see [28] for full list) for the outside dimensions of the MFBB: for smaller chips 15x15mm or a multiple of 15mm such as 15x30mm. For larger chips, the standard includes outer dimensions of 75x25mm, 75x50mm and 84x54mm. These large sizes are similar to the already common formats such as the microscopy slide, or the credit card in the microfluidic world.

The pitch, as can be seen in figure 3.1, is also chosen to be compatible with already currently used formats (e.g. microtiter plate) in the microfluidic world, while still trying to obtain a small pitch so that high interconnect applications are possible.

Besides fluidic interconnects, a microfluidic system sometimes needs an interconnect which is different than a fluidic one. Electrical and optical interconnects are typical examples. In the electronic field, there are already plenty of standards and products available as it is a much more mature market. The guidelines also recommend using these standard products for example connectors and spring loaded probes, but to group the interconnects in a specific area on the MFBB.

Figure 3.1 - Standardized dimensions: a. MFBB outline dimensions, b. Grid starting position, c. Grid pitch and d. Port annotation and preferred (bold) port positions., e. Cross section of figure d in a typical usage scenario, where the MFBB is clamped to the FCB and the seal between them is facilitated by an O-ring.

3.4 Methods

The above paragraphs describe a new way of designing microfluidics and the corresponding necessary standardization, which the MFManufacturing consortium [25] is attempting to realize. In this chapter, the focus is on various parts needed to design according to this new method, with a focus on the typical auxiliary parts used in a microfluidic system: inlet reservoir, pump, flow and pressure measurement and interfacing. Our approach will also stay true to the Lab on a Chip concept, rather than the Chip in a Lab which is currently often seen. To be able to design with this functionality driven approach a small part of a library of basic building blocks is proposed in Table 3.2. For six of the MFBBs, a more de-

tailed description, including fabrication details and device characterization tests, are given in the following paragraphs.

3.4.1 Differential pressure sensor MFBB

The pressure sensor building block (as shown in Table 3.2A) is a package to connect to a Honeywell differential pressure (24PCAFA6D). This package makes it possible to fit this sensor to a FCB using a standardized interface. Together with a hydraulic resistor in the FCB (e.g. a simple channel), this building block can also serve as a flow sensor by measuring a differential pressure drop across this channel.

The material of choice for these building blocks is a COC (Topas grade 6013, Axxicon, The Netherlands). This material is chosen because of its chemical resistance to a wide range of chemicals and the opportunity to scale up production applying methods such as hot embossing or even roll-to-roll hot embossing. This provides the ability to suit applications that will be subject to high chemical constraints and higher production volumes in the future, while for quick prototyping micro-milling was used. To bond the four layers together, solvent assisted thermal bonding was used [30]. A PCB was mounted on top of the MFBB to provide electrical interconnection to the MFBB using a flat flex cable.

3.4.2 Clamping MFBB

To connect the building blocks for the FCB, several clamping connectors are developed. These clamps are screwed onto the FCB to fix the MFBB and ensure port alignment and compression of the O-rings to achieve an effective seal. Clamps A (table 3.2E) and B (table 3.2F) are used if fluidic connections are made between FCB and MFBB. Clamp C (Table 3.2G) is used if a direct fluidic connection to the MFBB or FCB via tubing is required. All clamps are fabricated by direct milling. The tubing used in combination with clamp C is connected using ferrules to form a tight fit to the MFBB or FCB.

3.4.3 Valve MFBB

CEA-LETI developed a pneumatic valve (see table 3.2B), consisting of an assembly of COC layers, including an EPDM diaphragm [31]. This valve is pneumatically actuated. The design is adapted to the end-user application (flow rate, dead volumes, and diaphragm material). Depending on the design, the flow rate can reach 50mL/min, and the pneumatic pressure to close the valve is engineered to be between 100 kPa and 500 kPa. The footprint (layout, I/O position)

is identical for all the valves.

3.4.4 Pump MFBB

The pump MFBB is based on the previously patented [32] oscillating rotary piston pump principle (see table 3.2D). This pump is manufactured using thermoplastic injection moulding using polymers and elastomers that can be adapted to the application.

3.4.5 Reservoir MFBB

The reservoir MFBB (see table 3.2C) is fabricated by milling a block of PMMA as a top holder for 1.5mL HPLC sample vials. This top block also contains holes for three needles; two of these needles puncture the septum of the vial to be able to apply pressure inside the vial and collect the resulting flow of liquid. The third hole is used for a blunt needle that fits onto tubing and connects the external pressure pump to the MFBB. A layer containing microfluidic channels is solvent-bonded to this top block to route the fluids or gases from these needles to the desired positions, as defined by the standard.

3.4.6 Reaction chamber MFBB

The reaction chamber is a custom 30x15 MFBB (see table 3.2H). The volume of the chamber, the filters and the embedded reagents (powders, beads...) are adapted to the application. Some designs integrate pneumatic valves (see table 3.2B). In the example, two 20 μ m stainless steel filters are embedded in the chamber and 50 μ m beads are packed between the two filters. The MFBB is composed of two COC layers (or three depending on the designs).

3.4.7 Fluidic circuit board

The FCB is always a custom part that fits a specific application and interconnects the building blocks in a specific way. Three different types of FCB are discussed with different levels of complexity.

3.4.7.1 Simple polymer-based FCB

This FCB was developed to test the pressure sensor MFBB, to evaluate how it functions as a flow sensor. This FCB is fabricated in a similar way to the MFBB and consists of two layers of Zeonor 1020R which contains milled cavities and channels. An assembled version of this FCB is shown in figure 3.3. The

channels milled into the first layer are closed off by the second layer by means of solvent bonding. The cavities milled in the top side of the second layer are open to accept the building blocks and to ensure accurate alignment between the channels in the FCB and those in the building block. The interconnect between the FCB and the building block is formed by standard Viton O-rings. There are cavities in the FCB to hold the O-rings in place.

3.4.7.2 Complex polymer-based FCB

This FCB shown in figure 3.2 incorporates integrated membrane valves for customized flow control to the MFBBs. These membrane valves can be pneumatically actuated to direct flow both from and to MFBBs attached to the FCB. This allows, for instance, the directing of fluids to a mixer chamber and hold these liquids inside the chamber during the mixing process before directing the fluids further. This FCB is fabricated in a similar fashion to that described for the simple FCB also using milling and thermal compression solvent bonding. What makes this FCB complex is that it consist of six layers including a membrane layer. Each layer consists of 1.5 mm clear polystyrene plates and a SEBS (styrene ethylene butylene ethylene) membrane layer. This SEBS membrane was precisely cut by a CO2 laser.

27

Figure 3.2 - FCB with integrated valves

3.4.7.3 Glass-based FCB

In some cases, polymers cannot be used due to their material properties. For one of these cases, a glass FCB is developed. This FCB consists of two borosilicate glass layers. Both glass layers have wet etched channels using two depths of 75 and 200 μm . After bonding of these two layers, the final channels will be approximately 150 μm and 400 μm in diameter. The top layer has powder blasted through-holes for top down access, where as in a second design included in the same batch, another FCB allows for direct capillary gluing inside the deep channels from the side. On the top surface of the FCB, platinum electrodes are sputtered to a thickness of 125nm, using a tantalum seed layer of 15nm to improve adhesion. These platinum electrodes are used to create a routing for the electrical actuation of the MFBB valves used in combination with this FCB design. The connection between these MFBBs and the FCB are made using wire bonds. This FCB thereby demonstrates both fluidic and electrical functionality by providing interconnects for both domains.

3.5 Test methods

3.5.1 Differential pressure sensor

The pressure sensor building blocks are characterized both as a pressure sensor and as a flow sensor. For both the pressure and flow characterization, a known pressure or flow is applied to the system by a pressure driven pump (Fluigent MFCS-4C, France). A flow sensor (Fluigent type L, France) was used in a control loop to obtain a reliable flow rate. While applying various flow rates to the system, the output signal of the building block is recorded with a custom-made Labview 2014 application and MyDAQ data acquisition system (National Instruments, The Netherlands). Both the pressure and flow are applied in a staircase pattern which was cycled four times. The pressure/flow of each step in the staircase pattern is kept constant at a plateau value for 30 seconds. Figure 3.3 shows the complete test system, which is based on the simple polymer FCB, and includes the flow sensor MFBB. It consists of four standardized building blocks connected serially, starting with an inlet block, followed by the differential pressure sensor, a blocking plate to allow for future extensions, and an outlet block.

Figure 3.3 - Complete system to measure flow in fluidic circuit board with mounted MFBBs.

3.5.2 Valves integrated in the complex polymer PCB

The valves integrated in the complex polymer-based FCB are tested using a pressure pump (Fluigent MFCS-EZ) fitted with an in-line flow sensor (Fluigent type L). The pressure to the valve control channel is varied from 0 to max 200kPa, while the resulting flow is recorded. This is repeated for three different pressures (40, 60, 80 kPa) applied to the reservoir holding the liquid flowing through the valve.

29

3.5.3 Valve MFBB

A valve MFBB is characterized using a custom Fluigent test platform including a two-channel pressure regulator and an in-line flow sensor (Fluigent type XL). The system is run through a dedicated LabVIEW (National Instrument) interface using the Fluigent SDK that provides a fully automated operation and data analysis. The pressure needed to actuate the valve is varied from 0 to 100 kPa in 5 kPa steps. For each step, the flow rate is recorded during a period of 1 s (10 points every 100ms) after waiting for 3 s to ensure the system is in a steady state condition. This operation is repeated for three fluid inlet pressures (25 kPa, 50 kPa and 100 kPa).

3.5.4 Pump MFBB

The pump MFBB is characterized using a measurement of the displacement volume in various backpressure conditions. The backpressure pressure is controlled using a closed container, the pump is actuated at a speed of 30rpm for a given number of cycles. The obtained fluid volume allows the measurement of the pump displacement. The test is repeated for several downstream pressures. The obtained displacement with a backpressure of 0kPa is normalized to 100%.

The efficiency of the pump is calculated based on the ability to sustain this displacement at higher backpressures.

3.5.5 Clamping MFBB

The same system as that shown in figure 3.3 is used for leak testing. The clamping MFBB was attached to the FCB using four bolts for each MFBB. A Viton O-ring, placed in a recess in the FCB, provides the seal between the MFBBs and the FCB. Only an inlet block was used, while the other ports on the FCB were capped by a blocking plate. To test for leaks, the system was filled with DI water before positioning the final blocking plate. The pressure at the inlet was applied by a pressure-driven pump (Fluigent MFCS-4C, France) in a range from 0 kPa to 600 kPa. A flow sensor (Fluigent type L, France) was placed in line to check if the flow remained at zero. The pressure was applied in a staircase pattern both upwards and downwards. The system was allowed to come to equilibrium before taking a flow measurement.

3.6 Results and discussion

Table 3.2 - Overview of building blocks with their characteristics

Building block with photo	Description	Most relevant characteristics
<p>A. Differential pressure sensor</p>	<p>A building block to measure pressure. Due to the differential nature of the measurement, a flow measurement is also possible, by measuring pressure drop over a length of channel</p>	<p>Flow characterization ($R^2=0.98928$), Error bars indicate the hysteresis over the 1 repetition of the reference pattern.</p>
<p>B. Pneumatic valve 15x15mm</p>	<p>A valve building block to conditionally route liquids in a microfluidic system. Controlled by pneumatic actuation.</p>	<p>Pressure needed to close MFBB valve. Line for visual guidance.</p>
<p>C. 1.5mL Fluid reservoir</p>	<p>A 1.5 mL fluid reservoir which can be used to actively push liquid through a microfluidic system by applying a regulated pressure above the liquid.</p>	<p>Internal Volume of 1.5mL</p>
<p>D. High volume pump</p>	<p>A pump capable of obtaining high flow rates even when a large back-pressure exists.</p>	<p>Pumping performance at constant actuation, with respect to changing backpressure.</p>

<div>E. 15x15mm clamp MFBB</div> <div> </div>	<p>A clamp to mount a MFBB with 15x15mm outer dimensions to the fluidic circuit board. Also compresses the O-ring between the FCB and the MFBB to facilitate sealing.</p> <p>This clamp is suited to mount devices shown in table 3.2A, B and C to a fluidic circuit board.</p>	<div><p>Burst pressure test clamping system</p> <table><caption>Data for Burst pressure test clamping system</caption><tr><th>Pneumatic pressure (kPa)</th><th>Flow (µl/min)</th></tr><tr><td>0</td><td>-0.5</td></tr><tr><td>100</td><td>0.5</td></tr><tr><td>200</td><td>-0.5</td></tr><tr><td>300</td><td>-0.5</td></tr><tr><td>400</td><td>-0.5</td></tr><tr><td>500</td><td>-0.5</td></tr><tr><td>600</td><td>0.5</td></tr></table><p>Top line for increasing pressure, bottom line for decreasing pressure. Line for visual guidance.</p></div>	Pneumatic pressure (kPa)	Flow (µl/min)	0	-0.5	100	0.5	200	-0.5	300	-0.5	400	-0.5	500	-0.5	600	0.5
Pneumatic pressure (kPa)	Flow (µl/min)																	
0	-0.5																	
100	0.5																	
200	-0.5																	
300	-0.5																	
400	-0.5																	
500	-0.5																	
600	0.5																	
<div>F. 15x30mm clamp MFBB</div> <div> </div>	<p>A clamp to mount a MFBB with 15x30mm outer dimensions to the fluidic circuit board. Also compresses the O-ring between the FCB and the MFBB to facilitate sealing.</p> <p>This clamp is suited to mount the device shown in table 3.2H.</p>	<p>Similar characteristics to 15x15mm clamp</p>																
<div>G. Fluidic seal 30x15mm clamp MFBB</div> <div> </div>	<p>A building block used to connect 10 individual tubes to a FCB at once. Elastomeric ferules are used to simultaneously facilitate sealing between the FCB and the tubes.</p>	<p>Similar characteristics to 15x15mm clamp</p>																
<div>H. Custom reaction chamber 30x15mm MFBB</div> <div> </div>	<p>Custom reaction chamber adapted to the application.</p>	<p>Highly application dependent</p>																

3.7 Characterization

3.7.1 Differential pressure sensor MFBB

Repackaging this commercial pressure sensor, to comply to the new standard, does not negatively impact its excellent performance. The pressure response still behaves highly linear. With the MFBB connected to the system shown in figure 3.3, its performance as a flow sensor was also evaluated. The figure in table 3.2A shows the sensor output voltage for varying flow rates. The output of the flow sensor is very linear ($R^2=0.98928$). However, a small deviation from perfect linear behaviour can be observed. This is probably caused by the fact that the reference flow sensor (Fluigent type L) was operating at low flow rates, outside of its optimum operating range. This suspicion is confirmed by checking the measured flow by the MFBB as a function of the applied pressure by the pump, we again see a highly linear trend.

3.7.2 High volume pump MFBB

The MFBB pump is able to displace 300 μ L per cycle, achieving flow rates up to 90mL/min. Another key feature is its self-priming, valve less, blocking nature: making the pump suited to particle loaded liquids. More importantly, no fluid flow through the pump is possible when the pump is not driven. This is an advantage when either high or low pressure must be maintained at the ports of the pump before or after pumping phases. The figure in table 3.2D shows a sustained displacement for various backpressures. The diaphragm pump is not able to sustain a constant displacement for the various backpressures. Moreover, the pump MFBB is also reversible as it behaves in exactly the same way when the actuation direction is reversed; the inlet becoming the outlet and vice versa.

3.7.3 Valve MFBB

The figure in table 3.2B shows the closure behaviour of the MFBB valve. The results shows that by applying sufficient pressure to the control line, the valve can be closed for all three input pressures. When pressures higher than the fluid pressure are applied to the control line, the flow is reduced.

3.7.4 Integrated FCB valve from the complex FCB design

Figure 3.4 shows the closure behaviour of the integrated valves in the complex FCB design. In figure 4, three lines are visible for various pressures (P_s) applied to the reservoir holding the liquid flowing through the valve. As expected, the

valves are able to close and stop the flow if sufficient pressure is applied to the control channels. A control pressure equal to the pressure applied to the liquid reservoir results in closure of the valve.

Figure 3.4 - Closing pressure of an integrated valve. Line for visual guidance.

3.7.5 Clamping MFBB

The figure in table 3.2E shows the O-ring seals between the FCB and MFBB up to a pressure of at least 600kPa. When the pressure is increased from 0 to 100 kPa there is a slight positive flow, whereas for the decreasing steps the opposite effect is seen. This can be explained by the air that was trapped in the system being compressed and relaxing, allowing liquid to flow into the system and out again.

3.7.6 Design considerations

The current state of standardization is compatible with frequently used fabrication technologies, including less accurate technologies like direct milling. This results in relatively large building blocks for microfluidic systems, with long channels to connect these blocks. Trade-offs between for example dead-volume and pressure drop over the channels need to be made. The various 90 degree

corners, the fluid encounters, traveling from the FCB to the MFBB and back can have unintended behaviour like bubble trapping, adding dead volume or mixing. These drawbacks of a modular platform are not necessarily a problem, by integrating sensitive parts in the MFBB and having robust input and outputs on the MFBB.

3.8 Conclusions

In this chapter, we have proposed a modular platform that uses standard microfluidic building blocks. Together with a rapid manufacturing processes, this demonstrates a unique platform for quick and straightforward research and development. This is especially important to reduce the existing gap for applications to bridge the lab-to-fab gap, by reducing the time-to-market. This is mainly achieved by separating the functional and physical design by using a top-down design approach. We have developed several building blocks with basic functionality including reservoirs, valves, flow and pressure sensors, and a high volume pump. During development materials were chosen to be compatible with quick prototyping fabrication, but also scalable to other manufacturing techniques to achieve higher volumes. We show how these building blocks can be combined by using a FCB. The use of standards, in both the MFBB and interconnects, allows straightforward and rapid development of early prototypes. Moreover, it demonstrates the combined use of a variety of fabrication technologies in several materials such as glass, polymers and silicon. The combined use of these is difficult to achieve in a traditional monolithic lab-on-chip device. In the future, we will develop more building blocks to extend our library. This library will be made available in 2017 through the Microfluidics Manufacturing website [25] and on-line through a marketplace [26] to enable broad adoption by the microfluidics community. The wide support of key European players that are active in both manufacturing, design and equipment for microfluidics is what will make the difference compared to earlier attempts at modular microfluidics design.

3.9 Reference

- [1] A. Manz, N. Graber, H.M. Widmer, Miniaturized total chemical analysis systems: A novel concept for chemical sensing, *Sensors Actuators B Chem.* 1 (1990) 244–248. doi:10.1016/0925-4005(90)80209-I.
- [2] G.M. Whitesides, The origins and the future of microfluidics, *Nature.* 442 (2006) 368–373. doi:10.1038/nature05058.

- [3] R. Mukhopadhyay, Microfluidics: On the Slope of Enlightenment, *Anal. Chem.* 81 (2009) 4169–4173. doi:10.1021/ac900638w.
- [4] H. van Heeren, Standards for connecting microfluidic devices?, *Lab Chip*. 12 (2012) 1022. doi:10.1039/c2lc20937c.
- [5] T.S.J. Lammerink, V.L. Spiering, M. Elwenspoek, J.H.J. Fluitman, A. van den Berg, Modular concept for fluid handling systems. A demonstrator micro analysis system, in: *Proc. Ninth Int. Work. Micro Electromechanical Syst.*, IEEE, 1996: pp. 389–394. doi:10.1109/MEMSYS.1996.494013.
- [6] C. González, S.D. Collins, R.L. Smith, Fluidic interconnects for modular assembly of chemical microsystems, *Sensors Actuators B Chem.* 49 (1998) 40–45. doi:10.1016/S0925-4005(98)00035-5.
- [7] B.L. Gray, D. Jaeggi, N.J. Mourlas, B.P. van Drieënhuizen, K.R. Williams, N.I. Maluf, G.T. a. Kovacs, Novel interconnection technologies for integrated microfluidic systems, *Sensors Actuators A Phys.* 77 (1999) 57–65. doi:10.1016/S0924-4247(99)00185-5.
- [8] C.G.J. Schabmueller, M. Koch, A.G.R. Evans, A. Brunnschweiler, Design and fabrication of a microfluidic circuitboard, *J. Micromechanics Microengineering*. 9 (1999) 176–179. doi:10.1088/0960-1317/9/2/318.
- [9] A. Wego, S. Richter, L. Pagel, Fluidic microsystems based on printed circuit board technology, *J. Micromechanics Microengineering*. 11 (2001) 528–531. doi:10.1088/0960-1317/11/5/313.
- [10] M. Rhee, M.A. Burns, Microfluidic assembly blocks, *Lab Chip*. 8 (2008) 1365. doi:10.1039/b805137b.
- [11] O. Strohmeier, M. Keller, F. Schwemmer, S. Zehnle, D. Mark, F. von Stetten, R. Zengerle, N. Paust, Centrifugal microfluidic platforms: advanced unit operations and applications, *Chem. Soc. Rev.* 44 (2015) 6187–6229. doi:10.1039/C4CS00371C.
- [12] P.K. Yuen, SmartBuild—A truly plug-n-play modular microfluidic system, *Lab Chip*. 8 (2008) 1374. doi:10.1039/b805086d.

- [13] P.K. Yuen, J.T. Bliss, C.C. Thompson, R.C. Peterson, Multidimensional modular microfluidic system, *Lab Chip*. 9 (2009) 3303. doi:10.1039/b912295h.
- [14] P.K. Yuen, A reconfigurable stick-n-play modular microfluidic system using magnetic interconnects, *Lab Chip*. 16 (2016) 3700–3707. doi:10.1039/C6LC00741D.
- [15] K. Vittayarukkul, A.P. Lee, A truly Lego®-like modular microfluidics platform, *J. Micromechanics Microengineering*. 27 (2017) 035004. doi:10.1088/1361-6439/aa53ed.
- [16] K.A. Shaikh, K.S. Ryu, E.D. Goluch, J.-M. Nam, J. Liu, C.S. Thaxton, T.N. Chiesl, A.E. Barron, Y. Lu, C.A. Mirkin, C. Liu, A modular microfluidic architecture for integrated biochemical analysis, *Proc. Natl. Acad. Sci.* 102 (2005) 9745–9750. doi:10.1073/pnas.0504082102.
- [17] L.J. Millet, J.D. Lucheon, R.F. Standaert, S.T. Retterer, M.J. Doktycz, Modular microfluidics for point-of-care protein purifications, *Lab Chip*. 15 (2015) 1799–1811. doi:10.1039/C5LC00094G.
- [18] K.C. Bhargava, B. Thompson, N. Malmstadt, Discrete elements for 3D microfluidics, *Proc. Natl. Acad. Sci.* 111 (2014) 15013–15018. doi:10.1073/pnas.1414764111.
- [19] P. Loskill, S.G. Marcus, A. Mathur, W.M. Reese, K.E. Healy, μ organo: A Lego®-like plug & play system for modular multi-organ-chips, *PLoS One*. 10 (2015) 1–13. doi:10.1371/journal.pone.0139587.
- [20] J. Wissink, MATAS: A modular assembly technology for hybrid uTAS, (2000). <http://www.lionixbv.nl/download/pdf/MSTnews-feb-2000.pdf> (accessed August 4, 2014).
- [21] Ferrules for micro-fluidic connectors, (n.d.). <http://epigem.co.uk/technology/microfluidics/connectors> (accessed September 3, 2016).
- [22] Y. Fintschenko, Education: A modular approach to microfluidics in the teaching laboratory, *Lab Chip*. 11 (2011) 3394. doi:10.1039/c1lc90069b.
- [23] S. Miserendino, Y.-C. Tai, Modular microfluidic interconnects using

photodefinable silicone microgaskets and MEMS O-rings, *Sensors Actuators A Phys.* 143 (2008) 7–13. doi:10.1016/j.sna.2007.07.019.

[24] M. Gilde, H. van den Vlekkert, H. Leeuwis, A. Prak, Modular Design Approach for MicroFluidic Systems, in: *Tech. Proc. 2005 NSTI Nanotech-nol. Conf. Trade Show, 2005*: pp. 684–687. www.nsti.org/publications/Nano-tech/2005/pdf/1201.pdf (accessed October 3, 2017).

[25] MFManufacturing Project, (n.d.). <http://mf-manufacturing.eu/> (ac-cessed October 28, 2016).

[26] www.makefluidics.com, (n.d.). <http://www.makefluidics.com/> (accessed March 15, 2017).

[27] IWA 23:2016 - Interoperability of microfluidic devices -- Guidelines for pitch spacing dimensions and initial device classification, n.d. http://www.iso.org/iso/home/store/catalogue_tc/catalogue_detail.htm?csnumber=70603.

[28] H. van Heeren, T. Atkins, N. Verplanck, C. Peponnet, P. Hewkin, M. Blom, W. Buesink, J.-E. Bullema, S. Dekker, Design Guideline for Microflu-idic Device and Component Interfaces (part 1) ver. 2, 2016. doi:10.13140/RG.2.1.1698.5206.

[29] H. van Heeren, D. Verhoeven, T. Atkins, A. Tzannis, H. Becker, W. Beu-sink, P. Chen, Design Guideline for Microfluidic Device and Component Inter-faces (Part 2) ver. 1.2, 2016. doi:10.13140/RG.2.1.3318.9364.

[30] I.R.G. Ogilvie, V.J. Sieben, C.F. a Floquet, R. Zmijan, M.C. Mowlem, H. Morgan, Solvent processing of PMMA and COC chips for bonding devices with optical quality surfaces, *14th Int. Conf. Miniaturized Syst. Chem. Life Sci.* (2010) 1244–1246.

[31] N. VERPLANCK, N. SARRUT, F. BOIZOT, M. ALESSIO, FLUID CARD COMPRISING AT LEAST ONE FLUID VALVE, WO2017029348 (A1), 2017. [https://worldwide.espacenet.com/publicationDetails/bib-lio?F{T}=D&date=20170223&DB=EPODOC&locale=fr_EP&CC=WO&N-R=2017029348A1&KC=A1&ND=4](https://worldwide.espacenet.com/publicationDetails/biblio?F{T}=D&date=20170223&DB=EPODOC&locale=fr_EP&CC=WO&N-R=2017029348A1&KC=A1&ND=4) (accessed March 16, 2017).

[32] A. WATTELLIER, C. DEHAN, ROTARY-OSCILLATING SUB-ASSEMBLY AND ROTARY-OSCILLATING VOLUMETRIC PUMPING DEVICE FOR VOLUMETRICALLY PUMPING A FLUID, WO2015011384, 2015. <https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2015011384&recNum=1&maxRec=&office=&prevFilter=&sortOption=&queryString=&tab=PCT+Biblio> (accessed March 14, 2017).

Chapter 4

Tools to design and simulate microfluidics

In the history of the development of electronics, both standardization and design (software) tools played a large role. This enabled the complex electronics we know nowadays. In this chapter an integrated design tool is presented for the microfluidic field, with features similar to electronics design tools. Important features are a building block library and the system level spice simulations. The system level simulations are made possible by using spice models based on hydraulic resistance and buffers. They use less computation time than e.g. finite element models, which are commonly used in microfluidics. As a result quick design integration is possible, reducing the overall development efforts needed for a microfluidic system.

4.1 Introduction

In the previous chapter, it was argued that there is a clear need for the development of standardized and modular building blocks that can be fabricated at high volumes. Looking back at the development of the electronics industry, some important factors for successful development can be identified.

Figure 4.1 - Development of the electronics industry, demonstrating various milestones related to the ever increasing complexity over time.

The electronics field has made tremendous progress, especially since the invention of the transistor in 1947 by Shockley, Badeen and Brattain at Bell Labs. The transistor enabled the miniaturisation of electronic circuits. As a result the complexity of circuitry quickly evolved from a typical spider-assembly to more complex printed circuit board assemblies, enabled by the definition of standards by the IPC [1] in 1957. Additionally, the definition of the “complementary metal-oxide semiconductor” (CMOS) [2] standard around the early 1960's gave rise to the use of integrated circuits (ICs). Simultaneously, various (software) tools were developed to ease the task designing and routing schematics. A milestone in that area was set in 1973 with the release of SPICE [3], a simulation program originally developed at Berkeley to conduct various types of linear and non-linear circuit analysis. All these developments have resulted in various complex electronic circuits, e.g. demonstrated by the latest AMD Epyc server CPU's containing more than 19 billion transistors on a single die, operating at max. 3.2 GHz clock frequency. At these high frequencies, transferring information and making many electrical connections between e.g. CPU and memory becomes

problematic, resulting in the integration of several dies in a single device, the so called system-on-a-chip.

As one can imagine, designing a circuit with that many components is very challenging, and only possible with the aid of design software. In electronics, several packages are commercially available to help with the design, simulation, and layout of both CMOS ICs, as well as printed circuit boards. Often these software packages have extensive libraries of functional components, manufacturing standards, and simulation models.

An integrated tool that can do a similar job would be a most welcome addition to the microfluidics field. However, the multi-domain nature and broad range of applications of the field makes it difficult to provide a solution that is versatile enough to be accepted by a broad community of users. At present, simulations are often carried out in complex finite element packages such as COMSOL, Ansys Fluent, and many others. These type of simulations are very computationally intensive, and therefore require time, money, and expert knowledge. Alternatively, system level simulations are possible using electronic equivalent circuits, based on hydraulic resistance. This approach is much more suited to elaborate assemblies of (standardized) fluidic components, but only works if the flow is laminar, and the viscosity of the fluid involved is known.

In the remainder of this chapter, we describe a software package developed by PhoeniX software to design, layout and simulate modular fluidic systems based on a system level approach using linear models. We demonstrate that a complete microfluidics system can be simulated with minimal computation time. The typical workflow is explained using a flow sensor application as example. As a result, the time from concept to physical design has also decreased significantly.

4.2 Tools

One of the aims of the MFManufacturing project was to develop new software tools for microfluidic design. This followed from the need for a design and simulation tool to work with the newly defined standard [4–6]. This standard encompasses modular microfluidic design and therefore needed interconnects between the modules. The available software packages from the electronics field demonstrates the strength of integrating component libraries into the design tools. Printed circuit board design packages like KiCAD, Altium Designer and Circuit Design Suite (includes Multisim and Ultiboard) are much more than layout packages only. They provide aid during the whole design process, from

schematic design, simulation, board layout, to a final 3d model of the electronics board.

PhoeniX Software developed a complete software package to aid the design of a microfluidic system, within the framework of the ENIAC Joint Undertaking Microfluidic Manufacturing project. This software is based on their Optodesigner, originally intended for photonics. Within the MFManufacturing project standards [4–6] were developed for the interfacing of different components in a modular microfluidic system. The software is developed to make designing a system according to the standard more straight forward. Table 4.1 shows a typical top down design process, which starts by capturing the intended design. The second step is a verification, to check if the design is performing as intended. Often mathematical models are used to predict the behaviour of the design in a simulation. If everything works as expected, a final step is to implement the design in a physical way.

Table 4.1 - Complete system design, differences and similarities between electronics and microfluidic design

	Design intent	Simulation or design verification	Physical design
Typical PCB design package		$R = \frac{\rho L}{A}$ $C = \frac{\epsilon_r \epsilon_0 A}{d}$
Phoenix Software for microfluidics	<code>pressureSensor.place (location, clamp on/ off);</code>	$R_{hyd} = \frac{f_D \rho L Q}{2 D_H A^2}$ $C_{hyd} = \frac{V^*}{p^*}$ <p>See table 4.2 for more details</p>

One element that is powerful in these computer aided design tools are the libraries. If a component is chosen from the library during the design phase, a lot of information can already be loaded. This can for example be a mathematical model needed for the simulation of that component, the footprint of the component, and more recently some design suits also include supplier information like stock availability.

Phoenix's Optodesinger has a script based editor to let the designer put the design into the software. With the script, microfluidic building blocks can be loaded from a library. When a microfluidic building block is loaded, two sets of additional data are loaded: simulation information and physical dimensions. The first set includes the linear mathematical models that can be used in Ngspice to simulate the behaviour of the system. The second set includes the physical dimensions and contains both information about the block itself and of features in other layers. For example, a building block needs to be clamped to the fluidic circuit board. This building block will contain information where the screw holes and sealing features should be placed in the fluidic circuit board. To keep the libraries up to date with new building blocks, the software contains a possibility to synchronize with an online repository. An example of such an online repository is the makefluidics.eu [7] website containing components that comply to the new standard.

4.3 Methods

4.3.1 Front-end software use

To show the usefulness of simple linear simulations during the design phase of a microfluidic system, an example design will be used in the rest of this chapter to demonstrate the capabilities. A schematic overview is shown in figure 4.2a. This example system is a test bench to characterize valves. Parameters like opening and closing time, burst pressure, and hydraulic resistance are tested. To obtain these parameters both the flow through the valve and the pressure at the location of the valve need to be measured. Moreover, the test bench should not interfere with the behaviour between pump and valve, meaning that the pressure drop over the test bench should be neglectable.

A schematic overview of system functionality is given in figure 4.2a. Two differential pressure sensor microfluidic building blocks are used, one to obtain information about the flow rate and the other to obtain a pressure measurement. Furthermore, inlet and outlet blocks are needed to respectively connect a pump and a valve. To connect all components together, a fluidic circuit board is used. The design for this microfluidic system is made in the Optodesigner software introduced earlier. This software features the possibility to directly import these building blocks from a library. This is done in a script based fashion, shown in the code box below.

```
UTwente_MFM_PressureSensor() pressureSensor;
pressureSensor.place(in0->block@Y19+[0,0,90]:&microMillingFCB,&pClamp);

UTwente_MFM_PressureSensor() pressureSensor1;
pressureSensor1.setShowClamp(1); //before .place to get the topview right
pressureSensor1.place(in0->block@Y39+[0,0,90]:&microMillingFCB,&pClamp);

UTwente_MFM_PumpAdapter() converter;
converter.place(in0->block@F10+[0,0,90]:&microMillingFCB,&pClamp);

UTwente_MFM_LidAdapter() converter1; converter1.setShowClamp(1);
converter1.place(in0->block@F44+[0,0,90]:&microMillingFCB,&pClamp);
```

In the Optodesigner script above, the first two instances of the UTwente_MFM_PressureSensor are placed. One of the nice features of using a standardized platform is the ability to place components on a grid by a designator. The designator @y19 in the second line indicates that the centre of the first pressure sensor is placed at row y and column 19 resulting in the following coordinates, with an outline to grid spacing of 3mm and a pitch of 1.5mm, y-axis: $3+1.5*25(y)=40.5\text{mm}$ and x-axis: $3+1.5*19=31.5\text{mm}$. Also the inlet and outlet

blocks are placed, respectively at grid positions F10 and F44. To complete the design, fluidic channels need to be routed in the fluidic circuit board to connect the building blocks.

```
ml::UTwente_MFM_StraightChannel(in0->bend2@out0,out0->outlet@out0:7500.0,wch,hch) str6;  
ml::UTwente_MFM_StraightChannel_DeadEnd(in0->tjunc1@out1,out0->block@Y13:17900.0,wch,hch) str7;
```

Optodesigner script is used to route these channels. As can be seen above, parameters are used to indicate start position on the grid, length, channel width and height. These parameters are also used as input for the simulation. Depending on the type of feature placed, a different simulation model is loaded. A channel will for example be implemented as a resistor. However, a dead end channel will hold compressible air and is thus equivalent to a capacitor in the electrical domain. This will be explained in more detail in the next section.

Figure 4.2b shows the graphical layout resulting from running the script. The different colours in the drawing indicate different layers. These different layers are needed to physically fabricate the features in a material. The channel of this design will be milled in a piece of Cyclic Olefin Copolymer and have cross-sectional dimensions of 200x200 μm . The channel height of the indicated with R2 is varied to obtain the correct hydraulic resistance. Another particularly useful feature is the ability to store this physical layout data in the building block library. For example dimensional and positional data for an O-ring pocket in the fluidic circuit board can already be stored in the building block library. Note that the annotations of Rx, Cx in figure 4.2b for the different channels are not actually present in the design, but are added here for clarification purposes.

Figure 4.2c shows the electrical equivalent of the microfluidic design, values of the components are automatically calculated based on the parameters entered while placing the building block in the design. The automated nature of this process makes it easy to simulate the behaviour of the whole system, improving the ease of system integration of several building blocks.

Figure 4.2 - a) Schematic overview of the system, showing the 4 microfluidic building blocks used together with their functions. b) Output of the Optodesigner software, showing a design with 4 building blocks. In the top left corner an inlet block, on the bottom two pressure sensors. One to measure flow, the other to measure pressure. Finally, in the top right corner an outlet block where the liquid leaves the system. c) Equivalent electrical circuit used for simulation.

4.3.2 Mathematical, back-end software implementation

Optodesigner uses Ngspice to perform the simulation. This means the fluidic building blocks need spice models in order to be able to run a simulation. The channels filled with liquid have a certain hydraulic resistance and are thus modelled as their electrical equivalent, a resistor. The Darcy-Weisbach equation is used to relate the flow through the channel to the pressure across it, see table 4.2 for all parameters involved. The dead end channels are modelled as an electrical capacitance, this is a good match due to the compressibility of air. The ideal gas law is used to give an equation for the capacitance of the dead end channels.

Table 4.2 - Models used for simulation

FCB channel rectangular [8]	FCB dead end channel [9]
$R_{hyd} = \frac{\Delta P}{Q} = \frac{f_D \rho L Q}{2 D_H A^2}$ <p>Where f_D: Darcy friction factor ρ: density L: channel length Q: volumetric flow rate D_H: wetted diameter of channel A: cross-sectional area channel</p> <p>For a rectangular channel [8]: $f_D = 96 \frac{(1 - 1.3553\alpha + 1.9467\alpha^2 - 1.7012\alpha^3 + 0.9564\alpha^4 - 0.2537\alpha^5) A \eta}{\rho Q D_H}$ <p>Resulting in: $\frac{\Delta P}{Q} = 96 \frac{(1 - 1.3553\alpha + 1.9467\alpha^2 - 1.7012\alpha^3 + 0.9564\alpha^4 - 0.2537\alpha^5) \eta L}{1 \cdot 2 D_H^2 A}$ <p>Where α: w/h or h/w, respectively for smaller width or height η: Dynamic viscosity L: channel length D_H: $\frac{2wh}{w+h}$ $A = wh$</p></p> </p>	$C_{hyd} = -\frac{dV}{dp}$ <p>Ideal gas law for isothermal process: $V(t)p(t) = V^*p^*$</p> <p>Where V^*: Volume at t=0 p^*: Pressure at t=0</p> <p>Resulting in: $C_{hyd} = \frac{V^*p^*}{p(t)^2}$ <p>For small pressure deviation $p(t) \approx p^*$ giving: $C_{hyd} = \frac{V^*}{p^*}$ <p>Where $V^* = whl$ $p^* = 101 kPa$</p> </p></p>

4.4 Results and discussion

In the design, a differential pressure sensor microfluidic building block is used to measure the flow through the system. The range of pressures that can be measured is determined by the silicon die used in the microfluidic building block, but in this case ranges from 0 to 6 kPa. To achieve a good match between the measurement range of the differential pressure sensor and the used flow rates, the hydraulic resistance denoted by R2 in figure 4.2b needs to be tuned. Quick simulations of the system makes this tuning process easy. The design is already

loaded into the software, thus by changing the parameters of the features placed in the design, the flow pressure relations in the system are changed.

Figure 4.3 shows several results obtain by running a spice simulation from within Optodesigner. All results (except figure 4.3b) are obtain by using a flow source set to $100\mu\text{L}/\text{min}$. Figure 4.3a shows that the total pressure drop over the system stays below 6kPa. This is particularly useful with a weak pump, since the test bench doesn't apply a large load.

Figure 4.3b gives the most relevant data obtained from R2. The pressure drop over this section of channel should reach figures similar to the range of the pressure sensor at the highest flow rates. This is achieved by varying the height of the channel denoted by R2 in figure 4.2b. For the pressure sensor with a range of 6kPa the channel with a depth of $70\mu\text{m}$ is most suited.

Figure 4.3d shows a transient simulation of the system using a channel depth at location R2 of $70\mu\text{m}$. The build-up of pressure at $t=0$ to 100ms is caused by the compression of air in the dead-end channels to the pressure sensor. Within 100ms the system reaches equilibrium, making this system suited for characterization of slow closing valves. Figure 4.3e is another transient simulation using a smaller channel height of $30\mu\text{m}$. The response of the system has slowed down significantly, as the simulated system doesn't reach equilibrium within 500ms. Such a slow response is unacceptable for determining the speed of the valves under test.

In summary we are able to determine that the valve characterization platform is able to measure a flowrate of $100\mu\text{L}/\text{min}$, can be placed in series between the pump and the device under test due to a low total pressure drop. To characterize the switching behaviour of the valve under test, care has to be taken as the system itself has a response time in the order of 100ms. All this information was available a few seconds after the design was complete, just by pressing the simulation button. Showing the strength of the combination of a library of containing microfluidic building blocks, but also a simple simulation model making it possible to predict how the entire system will interact.

However, there are some pitfalls as the models that are being used are simple. Temperature could be an important factor. For the models it is assumed to be constant, while in practice temperature changes will affect the viscosity of the liquid. An increase of pressure is also to be expected in the dead end channels

if the temperature of the air inside increases. Furthermore, the currently used capacitance model is not suited at higher pressure values for two reasons. Firstly, simplifications are made during the derivation of the formula for the capacitance that are not valid for larger pressure variations. Secondly, the volume of the gas will become small when the pressure increases, meaning the liquid will travel into the channel. This additional length of channel will also add hydraulic resistance to the system, which is not taken into account at this moment. Moreover, it is assumed that the channels are already filled with liquid, while in practice you often start with air in the channels.

Figure 4.3 - a) Pressure at the input when applying a $100 \mu\text{L}/\text{min}$ flow through the system (measure channel height $70 \mu\text{m}$). b) Pressure drop over the measure resistance, while varying channel height. c) Schematic layout of the system d) Transient response of the system with a measure channel depth of $70 \mu\text{m}$ at three position in the system when applying a flow of $100 \mu\text{L}/\text{min}$. e) same as d), but with a measure channel depth of $30 \mu\text{m}$.

4.5 Conclusion

With the new microfluidic design software, developed in the MFManufacturing project, it is possible to quickly conduct system level simulations. Furthermore, the design can be set up quickly by placing MFBBs from a library on a standardized grid. The result can be checked by simulation subsequently, listing the pressure and flow at multiple positions in the system. Both steady state and transient responses can be obtained with these simulations. The design can be checked fast, due to the low computation time requirements of the spice simulations. Parameters can be varied to find the optimal performing design. Overall, the design tool provides a quick way to assemble a microfluidic system with standardized MFBBs which can be loaded into the software from a library.

4.6 References

- [1] History of IPC's Name, (n.d.). <http://www.ipc.org/ContentPage.aspx?pageid=IPCs-Name%0A> (accessed August 8, 2018).
- [2] F.M. Wanlass, Low stand-by power complementary field effect circuitry, 1963. <https://patents.google.com/patent/US3356858A/en> (accessed August 8, 2018).
- [3] The Spice Home Page, (n.d.). <http://bwrcs.eecs.berkeley.edu/Classes/IcBook/SPICE/> (accessed August 8, 2018).
- [4] IWA 23:2016 - Interoperability of microfluidic devices -- Guidelines for pitch spacing dimensions and initial device classification, n.d. http://www.iso.org/iso/home/store/catalogue_tc/catalogue_detail.htm?csnumber=70603.
- [5] H. van Heeren, T. Atkins, N. Verplanck, C. Peponnet, P. Hewkin, M. Blom, W. Buesink, J.-E. Bullema, S. Dekker, Design Guideline for Microfluidic Device and Component Interfaces (part 1) ver. 2, 2016. doi:10.13140/RG.2.1.1698.5206.
- [6] H. van Heeren, D. Verhoeven, T. Atkins, A. Tzannis, H. Becker, W. Buesink, P. Chen, Design Guideline for Microfluidic Device and Component Interfaces (Part 2) ver. 1.2, 2016. doi:10.13140/RG.2.1.3318.9364.
- [7] www.makefluidics.com, (n.d.). <http://www.makefluidics.com/> (accessed March 15, 2017).

[8] R.K. Shah, A.L. London, Laminar flow forced convection in ducts : a source book for compact heat exchanger analytical data, Academic Press, 1978. <https://www.sciencedirect.com/science/book/9780120200511> (accessed March 5, 2018).

[9] H. Bruus, Theoretical Microfluidics. 2008, New York Oxford Univ. Press. (2008). <http://scholar.google.com/scholar?hl=en&btnG=Search&q=in-title:Theoretical+Microfluidics#0> (accessed October 31, 2014).

Chapter 5

From chip-in-a-lab to lab-on-a-chip: a portable coulter counter using a modular platform

The field of microfluidics has been struggling to obtain widespread market penetration. In order to overcome this struggle, a standardized - modular platform is introduced and applied. By providing easy-to-fabricate modular building blocks which are compatible with mass manufacturing, we decrease the gap from lab-to-fab. These standardized blocks are used in combination with an application specific fluidic circuit board. On this board, electrical and fluidic connections are demonstrated by implementing an alternating current coulter counter. This multi purpose building block is reusable in many applications. In this study it identifies and counts 6 μm and 11 μm beads. The system is kept in a credit card sized footprint, as a result of in-house developed electronics and standardized building blocks. We believe that this easy-to-fabricate, credit card sized, modular and standardized prototype brings us closer to clinical and veterinary applications, because it provides an essential stepping stone to fully integrated point of care devices.

In press at Microengineering and Nanosystems as: S. Dekker, P.K. Isgor, T. Feijten, L.I. Segerink, M. Odijk, From chip-in-a-lab to lab-on-a-chip : a portable coulter counter using a modular platform, doi: 110.1038/s41378-018-0034-1.

5.1 Introduction

After the initial hype in microfluidics almost three decades ago, which occurred after the introduction of the micro total analysis systems (MicroTAS), the field of microfluidics continues to struggle with obtaining widespread market penetration [1,2]. Lack of standards, focus and communication between academics and industry could be the reason for this struggle [3]. Although many lab-on-a-chip devices are presented in the literature, the term chip-in-a-lab fits better with prototypes currently demonstrated in the microfluidic field [4]. Even though there are a few frontier commercialized devices (Abbott i-STAT and DNA PCR machines), most lab-on-a-chip devices are still stuck at a technology readiness level (TRL) of 3 or 4 (Figure 5.1). If these early prototype devices would become available commercially, they could already serve several niche markets and start to decrease the discrepancy between academic output and actual market revenue.

Figure 5.1 - Different stages of innovation. Diagram according to Centre for Process Innovation UK [5] and TRLs according to Horizon 2020 [6].

Currently, most of the microfluidic systems are not compact, due to issues with system integration [7]. Even though auxiliary equipment is part of microfluidic set-ups, it is originally intended for other fields, it is bulky and not easy to miniaturize [8]. Moreover, components for fluid control, visualization and signal detection are not interoperable with each other, thus commercialization is prevented [7,9]. In order to realize true point-of-care (POC) microfluidic devices, this bulky auxiliary equipment should be miniaturized. Decreasing and standardizing the footprint of auxiliary components will be a step in enhancing portability and reducing equipment dependability. Furthermore, it will improve system integration and compactness, ultimately preventing microfluidic systems to end up in

the valley of death between academia and industry.

Until now standards have not been established in the microfluidic field, which leads to adoption of equipment from other fields, e.g. smart phones for read-out [10], microscope slides, microtiter plates, and Luer connectors [11]. Therefore it is difficult to assemble them in one compact system, which often manifest itself as a so-called spider web assembly in the lab where many tubes and wires connect the various parts to each other. Moreover, the microfluidic community prefers a wide variety of substrate materials including glass, silicon and polymers, such as polydimethylsiloxane (PDMS). Even though PDMS is a popular material amongst researchers, it lacks high volume and low cost fabrication and does not have long shelf life, making it unsuitable for realizing commercial products [8,12,13]. In order to commercially benefit from already presented microfluidic devices in academia, which are based on a wide variety of materials, a standardized platform is needed. To provide successful system integration, a large consortium of major industrial and academic partners have introduced standardized elements that maintain compatibility and interoperability between various system parts. Most of the standards that are described in the ISO workshop agreement 23:2016 [14] and whitepapers [15,16] relate to footprint and interconnect positions of smaller microfluidic elements. In this study, microfluidic building blocks (MFBBs) with a standard footprint are prepared according to ISO workshop agreement and assembled on a fluidic circuit board (FCB). The modular platform combines multiple materials, including a glass coulter counter chip, plastic MFBBs, and a FCB that is fabricated using rapid prototyping. Instead of PDMS, using a polymer like cyclic-olefin copolymer (COC) provides high volume manufacturing while maintaining biocompatibility and FDA approval.

In this chapter, we reduce the gap between academia and industry one step closer by demonstrating the implementation of a commonly used technology on a standardized platform. The widely applicable alternating current (AC) coulter counter is chosen, since it supports label-free detection of single cells, detection of morphological defects and categorization of different cell types [17]. The possibility of performing cell sorting, cancer research and drug screening makes an AC coulter counter a favourable building block [18,19]. In this study, the AC coulter counter previously demonstrated by Segerink et al. [20] is combined with already available building blocks [21]. The resulting credit card sized, modular system provides label free, real-time detection of different sized beads. Elimination of auxiliary components is not yet possible. However, an in-house developed, portable and low cost lock-in amplifier based on a digital signal pro-

cessor (DSP) evaluation board with custom front-end increases the possibility of having it in a MFBB format in the future. The standardized MFBBs facilitate easy transition of this prototype to the market.

5.2 Results & Discussion

5.2.1 Fabrication results

Thanks to utilization of the standard described in ISO Workshop agreement 23:2016 [14], individual MFBBs that provide various functions are assembled onto a credit card size FCB (Fig. 5.2a and 5.2c.1). The printed circuit board (PCB) holds all necessary electronics needed for the pseudo-differential measurement and increases the compactness of the system (Fig. 5.2b). Interfacing and assembling MFBBs (Fig. 5.2c.2) onto the FCB (Fig. 5.2c.1) provides functionality and interconnection, respectively. Necessary tools for assembly are shown in Fig. 5.2c.3. These standardized parts can be put in an online repository to be easily integrated in future projects [22]. The standardization process together with MFBBs facilitates increasing the TRL (Fig. 5.1) and decreases the gap between the “chip in a lab” concept and a commercial point of care device.

Figure 5.2 - a) Assembled FCB for the particle counting. b) Electronics for the particle counting. c) Fluidic parts of the system: (1) FCB with integrated electronics, (2) MFBBs and (3) clamps to assemble the system, respectively.

5.2.2 Experimental results

The AC coulter counter chip integrated onto a FCB is used to detect 6 μm and 11 μm polystyrene beads in phosphate-buffered saline (PBS). Three sets of experiments are conducted. Solutions that contain only 6 μm beads, only 11 μm beads or both of them in 1:1 (v/v) ratio are pumped through the microfluidic channels. The results for these measurements are shown in Table 5.1. The video data is used to obtain the trajectory of the bead as well as the size of a bead, which is subsequently linked to the electrical signal. An exemplary result of this process is shown in Fig 5.3b. As Table 5.1 shows 6 μm beads were detected while running the 11 μm bead solution. Although the system in this case should only detect 11 μm beads as only those kind of beads are present in the bulk solution, some 6 μm beads are still detected from earlier experiments that are left behind in the channels. In order to calculate the detection rate, the number of video detection matched to an electrical signal peak is divided by the total amount of beads detected in the video.

Table 5.1 - Number of detected beads in the datasets: video, electrical, and combined.

Exp.	Video data Nr. of beads		Electrical data Nr. of peaks	Combined Nr. of video detections matched with electrical peaks					Average bead velocity
	6 μm	11 μm		6 μm		11 μm		False positives	
				#	Detection rate	#	Detection rate	#	
6 μm solution	173	0	164	148	86%	0		14	110 $\mu\text{m/s}$
11 μm solution	84	85	113	27	32%	84	99%	3	344 $\mu\text{m/s}$
Mixed solution	118	46	101	46	39%	46	100%	9	289 $\mu\text{m/s}$

The results show that both solutions that contain 11 μm beads have a detection rate of more than 99 percent. The detection of 6 μm beads was more challenging, since the peaks generated are close to the noise band (Fig. 5.3A). 1 mV_{rms} was deemed to be a suitable threshold for peak detection, since the root mean square (RMS) level of the noise was 0.307mV_{rms}. The largest contributor to the noise was the minimal shielding on the FCB. Even though the threshold was set to 3 times of the RMS level of the noise, some false positives still occurred. The threshold was kept fixed to prevent lowering the detection rate. This threshold resulted in detection rates of 86 %, 39 % and 32 % for 6 μm beads at velocities

of 110 $\mu\text{m/s}$, 289 $\mu\text{m/s}$, 344 $\mu\text{m/s}$, respectively. From these results it is clear that detection rate was negatively influenced by higher flow speeds, since higher flow speeds result in higher frequency components in the detected peaks. Depending on the filter setting in the lock-in amplifier, removal of these high frequency components leads to a peak amplitude below the threshold for 6 μm beads. Increasing the bandwidth of the filters to allow the higher frequency components to pass is not favourable as the noise would also increase.

Figure 5.3 - a) Differential impedance measurement with 6 μm and 11 μm beads. In total 101 beads were electrically detected, 46 of them are 6 μm , 46 of them are 11 μm beads and 9 of them are false positives. b) Impedance data of an individual 11 μm bead combined with the video frames used to obtain its trajectory. c) Histograms of the peak amplitudes for a mixed solution.

The distribution of detected peak amplitudes for the mixed solution is shown in Fig. 5.3c. The peak amplitudes for the 11 μm beads are between 1.9 mV_{rms} and 20.7 mV_{rms}, while the majority of peak amplitudes for 6 μm beads is between 1 mV_{rms} and 1.8 mV_{rms}, with an average of 1.6 mV_{rms}. Since the total amplification of the pseudo differential measurement electronics is 100 kV/A, it results in a 16nA current difference between the RC reference and the microfluidic chip. So a 6 μm bead passing the electrodes generates a difference of 0.3 % in the total current flowing between the electrodes. The wide distribution of the peak amplitudes for 11 μm beads (Fig. 5.3c) can be explained by size variation of the

beads, as well as their position in the non-uniform electric field between the planar electrodes. The coefficients of variation (CV) supplied by the manufacturer are 18 % and 7 % for the 11 μm and 6 μm beads, respectively (Fig. 5.4). This variation results in a small overlap of the populations. Due to this overlap, both populations can generate equal peak amplitudes. Moreover, the peak amplitude of the signal depends on bead volume instead of bead diameter. Therefore, the histogram in Fig. 5.3c has a broader distribution than the plot in Fig. 5.4. The two outliers in the 6 μm peak amplitude histogram are 6 μm beads which were falsely linked to an electrical peak of a 11 μm bead. This occurs when a 6 μm bead is not electrically detected and a 11 μm bead is present in the same 100 ms time interval.

Figure 5.4 - Size distribution (based on a normal distribution and mean/CV figures of the manufacturer) of beads in mixed solution, showing a slight overlap around 7 μm .

In order to detect particles smaller than 6 μm , a reduction of the noise level is needed. This can be realized by better shielding of the FCB. The presented system could be used for the detection and counting of cells with diameters in the range of 10 μm to 15 μm like white blood cells. Improvement in the noise level will enable bacteria and yeast cell detection which have sizes in the range of 1 μm to 6 μm .

5.3 Materials and Methods

5.3.1 Coulter counter chip

In this study, we combined the coulter counter chip that is demonstrated by Segerink et al. [20] with our standardized platform in order to pave the way for a POC device. This chip consists of coplanar electrodes that are fabricated in a microfluidic channel, as shown in Fig. 5.5a. An in-house developed lock-in amplifier is used to apply an alternating potential across the electrodes, resulting in an electric field inside the fluidic channel. The impedance of the system increases approximately 0.01%, when an insulating polystyrene bead passes through this electric field.

Figure 5.5 - a) Coulter counter chip with a detailed view of coplanar electrode pair used for detection. b) Inlet reservoir MFBB for bead solution. c) Outlet reservoir MFBB for waste disposal.

5.3.2 Design of the microfluidic system

A design philosophy was used where standardization plays a major role. The system consists of standardized MFBBs that provide the functionality of the system along with aforementioned coulter counter chip. A FCB is designed to connect these functional blocks. Interconnect positions between the MFBBs and the FCB are standardized. A footprint and interconnect grid for the MFBB are described in the design guidelines [14–16]. Thanks to this standardization approach, the MFBBs are reusable. Fig. 5.5a-c show the MFBBs used to assemble the microfluidic system: a coulter counter chip (Fig. 5.5a), a 1.5 mL reservoir containing the sample (Fig. 5.5b) and a polymethyl methacrylate (PMMA) block to connect tubing to a waste reservoir (Fig. 5.5c). An external pressure regulator (Fluigent MFCS-4C, France) is used to pump the bead solution through the system. Electronics is developed in-house to amplify and record the impedance signals generated in the coulter counter chip.

5.3.3 Detection and signal processing electronics

In order to detect the impedance signals that the coulter counter chip generates, a two-step solution is used. As stated before, only very small changes in impedance of approximately 0.01% generated by the passing beads needs to be detected. Sensitive electronics were developed, including a (pseudo-) differential measurement for detection. Fig. 5.6 shows a block diagram of this pseudo-differential set-up. A resistor and capacitor reference circuit was used to mimic the behaviour of the coulter counter chip. As a result, the small changes in impedance are recorded with more detailed in the dynamic range of the 14-bit

analog-to-digital converter (ADC) of the lock-in amplifier. When a bead passes by the electrodes, a difference in current through the coulter counter chip arises. The signal of interest is obtained by subtracting the coulter counter signal from the reference signal. The programmable gain amplifier matches this signal to the dynamic range of the input for the next stage. Additionally, an in-house developed digital lock-in amplifier is used to significantly reduce the amount of noise in the recorded signal. Moreover, the trans-impedance amplifier was placed close to the coulter counter chip on the FCB to prevent issues with noise. All other electronics are soldered on a PCB.

Figure 5.6 - Block diagram illustrating the pseudo-differential measurement. Parts within the dotted line are mounted on the PCB.

The in-house developed lock-in amplifier consists of three main parts: an ADC, a DSP and a direct digital synthesis (DDS) integrated circuit (Fig. 5.6). Both the ADC and DDS provide an interface between the analog domain and the digital domain. The DDS generates a sinusoidal signal that is applied to the coulter counter chip and the analog output signal of it is digitized by ADC. Moreover, the lock-in process is performed by a DSP to prevent additional noise. In this study, we used three MFBBs: a detection unit (coulter counter chip), an inlet reservoir, and an outlet reservoir. The inlet reservoir MFBB is a gas tight 1.5 mL high performance liquid chromatography (HPLC) vial which is connected to two syringe tips in a PMMA block with a standard footprint [21]. The coulter counter chip is fabricated out of two glass substrates, as described previously [20]. In the first glass layer microfluidic channels are etched, and fluidic inlet holes are powder blasted. On the second glass layer platinum electrodes are patterned by a recess etch, sputtering, and lift-off process. These two layers are fusion bonded together [20]. The outlet reservoir MFBB is a PMMA block with a standard footprint. A hole (3mm) is drilled to place Tygon tubing fitted around 1/16" Teflon tubing.

Figure 5.7 - Layout of the FCB providing fluidic and electrical connections between different microfluidic building blocks.

The FCB is fabricated using two plates of 2 mm thick cyclic olefin copolymer (COC) (DENZ BIO-Medical, Austria) (Fig. 5.7). COC is a good candidate for high volume manufacturing, because it can be injection moulded and is Food and Drug Administration (FDA) approved. The top side and bottom side of the top plate are processed for electrical connections and fluidic connections, respectively. Micromilling is used to fabricate fluidic channels and electrical traces. There are two microfluidic channels of different size in the FCB. One of them directs the fluid to the coulter counter chip (width: 200 μm) and the other one is used for bypassing (width: 800 μm). The depth of both fluidic channels is 200 μm , while the width and height of electronic traces are 400 μm . These dimensions are suitable for using a standard small outline integrated circuit (SOIC) package. For bonding, the surfaces of two COC plates are exposed to cyclohexane vapour for 3 min. and afterwards immediately pressed together for 15 min. at 2.1 MPa pressure at 110 $^{\circ}\text{C}$ (Carver 3889CEB.4NE1001). Mounting holes are drilled, in order to allow anchoring of MFBBs to the FCB using clamps. The grooves for the electrical traces are filled with Silver/Silver Chloride paste (Gwent C2051014P10) to provide electrical connections. Before the paste cures, electrical components are placed, eliminating the need to solder afterwards. O-rings between the MFBBs and FCB facilitate a fluidic seal. Pogo pins (Smiths Connectors, 101582) are used to obtain an electrical connection between the FCB and the coulter counter chip. A trans-impedance amplifier is mounted on the FCB and additional electronic circuitry is provided by a separate PCB, which is connected to the FCB with a card edge connector.

5.3.4 Experimental

6 μm bead (Polystyrene Red Dyed Microsphere, Polyscience Europe, Germany) and 11 μm bead (Copolymer Microspheres 7510A, Thermo Fisher, USA) solutions with a concentration of 106 beads/mL in phosphate buffered saline (PBS) (Sigma Aldrich, The Netherlands) are prepared. Tween 20 (Sigma Aldrich, The Netherlands) is added to both solutions with a volume fraction of 0.1% to prevent clustering of the beads. These solutions are thoroughly mixed before they are loaded into the inlet reservoir MFBB. In addition to a height difference of 1 cm between inlet and outlet reservoir, a pressure pump is initially used to introduce fluid into the channel. The current through the coulter counter chip is recorded at 0.3VRMS, 90 kHz in real-time using a LabView program interfacing the lock-in amplifier. The total amplification of the electronics is 100kV/A.

The recorded data is subsequently processed with a MATLAB (MathWorks, USA) script, in order to detect peaks that represent beads. A moving average filter is applied to the signal and the result of this operation is subtracted from the original signal to remove drift in the signal. Peak amplitudes and corresponding times are obtained from this processed signal and compared to the video data.

Video data is obtained during the experiment, using a microscope (Leica DMLM and SUSS MicroTec, Germany) and a high speed camera at 60 fps (Grasshopper 3, Point Grey, USA). A MATLAB script is used to analyse the resulting video. The script detects the amount of pixels the bead occupies, in order to identify different bead sizes. The location and size information for each bead is saved to determine the exact time that the bead passes the electrodes. Subsequently, this time is used to find the corresponding peak in the impedance data. Overall this script provides information about detected bead size, peak amplitude and bead velocity for each bead.

5.4 Conclusion

In this chapter, we demonstrate a low-cost, modular particle counting microfluidic system with a credit card sized footprint. The reusable standardized parts decrease the design and fabrication time, which leads to a reduction in the time-to-market for industrial development. The standard platform provides the possibility of integrating different materials in one system. Here, a COC, a PMMA and a glass device are combined. The low cost of in-house built electronics and lock-in amplifier facilitates a route to commercialization while still keeping system performance up to par. We achieve to measure a 0.3 % change in the

impedance signal using a pseudo-differential measurement. As a result, 6 μm and 11 μm polystyrene beads were differentiated. All 11 μm beads were electrically detected, while the detection rates for 6 μm beads were 86 %, 39 % and 32 % depending mostly on the flow rate used. Better control over this flow rate will ensure a higher detection rate in the future. Moreover, better shielding of especially the trans-impedance amplifier on the FCB will reduce the noise levels. Most importantly, we show that this standardized platform bridges the gap from “chip-in-a-lab” to “lab-on-a-chip”. The only drawback of the system is the use of an external lock-in amplifier and a pressure regulator. However, we are confident that we can shrink the size of these two components towards obtaining a commercial product.

5.5 References

- [1] A. Manz, N. Graber, H.M. Widmer, Miniaturized total chemical analysis systems: A novel concept for chemical sensing, *Sensors Actuators B Chem.* 1 (1990) 244–248. doi:10.1016/0925-4005(90)80209-I.
- [2] H. Becker, Hype, hope and hubris: the quest for the killer application in microfluidics, *Lab Chip.* 9 (2009) 2119. doi:10.1039/b911553f.
- [3] G. Salgado, *Barriers to the Diffusion of Microfluidics from Research to Market*, Catolica Lisbon, 2016.
- [4] P. Neužil, C.D.M. Campos, C.C. Wong, J.B.W. Soon, J. Reboud, A. Manz, From chip-in-a-lab to lab-on-a-chip: towards a single handheld electronic system for multiple application-specific lab-on-a-chip (ASLOC), *Lab Chip.* 14 (2014) 2168–2176. doi:10.1039/C4LC00310A.
- [5] The Innovation Phase - CPI, (n.d.). <https://www.uk-cpi.com/about/the-innovation-phase> (accessed April 4, 2018).
- [6] Technology readiness levels, (n.d.). http://ec.europa.eu/research/participants/data/ref/h2020/wp/2014_2015/annexes/h2020-wp1415-annex-g-trl_en.pdf (accessed March 15, 2018).
- [7] L.R. Volpatti, A.K. Yetisen, Commercialization of microfluidic devices, *Trends Biotechnol.* 32 (2014) 347–350. doi:10.1016/j.tibtech.2014.04.010.
- [8] M.I. Mohammed, S. Haswell, I. Gibson, Lab-on-a-chip or Chip-in-a-

lab: Challenges of Commercialization Lost in Translation, *Procedia Technol.* 20 (2015) 54–59. doi:10.1016/j.protcy.2015.07.010.

[9] C.D. Chin, V. Linder, S.K. Sia, Commercialization of microfluidic point-of-care diagnostic devices, *Lab Chip.* 12 (2012) 2118. doi:10.1039/c2lc21204h.

[10] V. Gubala, L.F. Harris, A.J. Ricco, M.X. Tan, D.E. Williams, Point of care diagnostics: Status and future, *Anal. Chem.* 84 (2012) 487–515. doi:10.1021/ac2030199.

[11] H. van Heeren, Standards for connecting microfluidic devices?, *Lab Chip.* 12 (2012) 1022. doi:10.1039/c2lc20937c.

[12] Y. Temiz, R.D. Lovchik, G. V. Kaigala, E. Delamarche, Lab-on-a-chip devices: how to close and plug the lab?, *Microelectron. Eng.* 132 (2014) 156–175. doi:10.1016/j.mee.2014.10.013.

[13] R. Mukhopadhyay, When PDMS isn't the best, *Anal. Chem.* 79 (2007) 3248–3253. doi:10.1021/ac071903e.

[14] IWA 23:2016 - Interoperability of microfluidic devices -- Guidelines for pitch spacing dimensions and initial device classification, n.d. http://www.iso.org/iso/home/store/catalogue_tc/catalogue_detail.htm?csnumber=70603.

[15] H. van Heeren, T. Atkins, N. Verplanck, C. Peponnet, P. Hewkin, M. Blom, W. Buesink, J.-E. Bullema, S. Dekker, Design Guideline for Microfluidic Device and Component Interfaces (part 1) ver. 2, 2016. doi:10.13140/RG.2.1.1698.5206.

[16] H. van Heeren, D. Verhoeven, T. Atkins, A. Tzannis, H. Becker, W. Buesink, P. Chen, Design Guideline for Microfluidic Device and Component Interfaces (Part 2) ver. 1.2, 2016. doi:10.13140/RG.2.1.3318.9364.

[17] T. Sun, H. Morgan, Single-cell microfluidic impedance cytometry: a review, (n.d.). doi:10.1007/s10404-010-0580-9.

[18] R.-J. Yang, L.-M. Fu, H.-H. Hou, Review and Perspectives on Microfluidic Flow Cytometers, *Sensors Actuators B Chem.* (2018). doi:10.1016/j.snb.2018.03.091.

- [19] Y. Xu, X. Xie, Y. Duan, L. Wang, Z. Cheng, J. Cheng, A review of impedance measurements of whole cells, *Biosens. Bioelectron.* 77 (2016) 824–836. doi:10.1016/j.bios.2015.10.027.
- [20] L.I. Segerink, A.J. Sprenkels, P.M. ter Braak, I. Vermes, A. van den Berg, On-chip determination of spermatozoa concentration using electrical impedance measurements, *Lab Chip*. 10 (2010) 1018. doi:10.1039/b923970g.
- [21] S. Dekker, W. Buesink, M. Blom, M. Alessio, N. Verplanck, M. Hihoud, C. Dehan, W. César, A. Le Nel, A. van den Berg, M. Odijk, Standardized and modular microfluidic platform for fast Lab on Chip system development, *Sensors Actuators B Chem.* 272 (2018) 468–478. doi:10.1016/j.snb.2018.04.005.
- [22] www.makefluidics.com, (n.d.). <http://www.makefluidics.com/> (accessed March 15, 2017).

Chapter 6

Standardized PARallelization of multichamber Chips, the SPARC platform

* Anke Vollertsen and Stefan Dekker provided equal contributions.

For pharmaceutical industry to fully benefit from Organ on a Chip technology some engineering challenges and biological challenges need to be overcome. For industrial use of Organ on a Chip systems they need to have high throughput capabilities, as large numbers of compounds need to be analysed. At the moment the high complexity of a single Organ on a Chip and high numbers of parallelization do not mix. The envisioned use of the presented high throughput platform are several applications, for example: screening of drug treatments on generic cell cultures or drug efficacy testing for personalized medicine. To realize such a platform some engineering challenges need solving, these are presented in this chapter: a standardized, modular platform for parallel screening. The system consists of three building blocks with 64-chamber each, resulting in a total of 192 individually addressable chambers. With SPARC the throughput per time unit can be increased threefold and it also increases versatility since the building blocks can also be operated independently of one another. The use of modular building blocks improves fabrication ease and yield. With food dye the individual addressing of the chambers is shown, hereby showing that this platform has a high potential for high-throughput screening.

This work will be presented in a modified form as a poster presentation at μ TAS 2018: Anke Vollertsen, Stefan Dekker, Britt Wesselink, Rob Haverkate, Johan Bomer, Hoon Suk Rho, Robert Jan Boom, Maciej Skolimowski, Marko Blom, Andries D. van der Meer, Robert Passier, Albert van den Berg, Mathieu Odijk. Standardized, Modular Parallelization Platform for Microfluidic Large-Scale Integration Cell Culturing Chips.

6.1 Introduction

Organ on a Chip is a hot topic in the microfluidic field, and with good reason. They have the potential to change healthcare as we know it. At present, the pharmaceutical industry could benefit from this technology, by replacing animal models with the more physiologically relevant Organ on a Chips. This could prevent failure of costly clinical trials in later stages of drug development. In the future Organ on a Chips derived from patient cells can even provide personalized medicine [1].

However to reach this utopian view on Organ on a Chip, the technology should be transferred from academia to industry. At the moment several start-up companies exist and are implementing Organ on a Chip devices commercially, however these products are not high-throughput [2]. One of the biggest needs of the industry is the testing of huge numbers of compounds at a variety of concentrations, requiring the ability of parallel testing.

To fulfil this need, automated high-throughput screening is needed, requiring large amounts of individually addressable compartments. At the moment PDMS is preferred by academics for the fabrication of their devices, due to its ease of use and rapid and easy fabrication. Furthermore, PDMS is an elastomer and therefore suited to create valve membranes which control access to individual chambers. Some systems have already been shown in literature: Gómez-Sjöberg et al. show a system with 96 chambers for automated cell culturing [3], and Blazek et al. show a system with 128 chambers to investigate cell-signalling events [4]. However, the use of PDMS limits the size of the chips that can be fabricated, due to shrinkage of the PDMS [5]. This in turn limits the number of compartments that can be realized in a single chip. To reach high-throughput screening both automation and standardization are important [6]. Standards ensure that interfacing with automated fluid handling equipment and eventually readout is possible. Furthermore, quick re-purposing of the platform is possible due to the reversible nature of the interconnects.

In this chapter, we focus on the engineering challenge of obtaining a high-throughput screening platform. The main requirement for the system is the availability of many individually addressable chambers, which can be used to create various experimental conditions. The engineering challenge is that a lot of control lines need to be implemented to obtain the individual addressing. This is followed by the challenge of fabricating a large device. A similar problem was encountered in the electronics industry in the late fifties and was termed “The tyranny of numbers”

by the then Vice President of Bell Labs [7]. Electronics at that time were getting so complex in the number of components that it was nearly impossible to get them connected reliably. In the electronics this was solved by the introduction of the printed circuit board. To prevent “The tyranny of numbers” from happening in the presented system a standardized microfluidic platform [8] which incorporates a fluidic circuit board (FCB) is used. A trade-off is made between the number of compartments and the size of the microfluidic building blocks (MFBBs) and therefore the fabrication ease and yield. In practice a MFBB with 64-chambers resulted in a layout complexity that gave acceptable yield. Additionally, a binary number provides an easy way (by splitting channels) to keep the flow resistance to each chamber equal. To still keep the total chamber count high, three of these 64-chambers MFBBs are combined into one system, resulting in a total of 192 chambers. The three MFBB are interconnected by means of a FCB, avoiding tubing. The FCB has sets of valves to control which of the MFBBs are connected to the shared control lines, which are connected to an external solenoid through tubing. The standard as described in the ISO international workshop agreement 23:2016 [8] also foresees in standardized outer dimensions for compatibility with external read-out equipment. A welcome advantage of the FCB and MFBBs combination is that faulty MFBB can be replaced while the rest of the system stays running. Furthermore, proven MFBBs can be use on another FCB to realize a new system, which can be beneficial for future body-on-a-FCB applications.

6.2 Design

In summary, the design consists of a standardized modular platform [8–10]. Functionality of the system is mostly provided by microfluidic building blocks. Instead of using tubing to connect the individual building blocks, a fluidic equivalent of a printed circuit board is used. The fluidic circuit board connects the three building blocks to auxiliary solenoid valves and provides the possibility to enable or disable one or more building blocks by means of integrated valves, hereby reducing the amount of external control lines needed.

6.2.1 64-chamber microfluidic building block

64 chambers are present in a three layer MFBB, and each chamber is individually addressable. This facilitates the creation of 64 different sets of experimental conditions. The addressability of the chambers is implemented with multiplexed valves, minimizing the number of necessary control lines. To control $N!/((N/2)!)^2$ chambers N control lines are needed [11]. Valves are created by

crossing channels in the control layer and fluidic layer [12]. The width of the channel in the control layer where it crosses the fluidic channel determines if the fluidic layer can be pinched off or not. The fluidic channel can be crossed by the control layer by using a narrow channel section, preventing full closure (fig. 6.1c). During operation of the valve, the thin membrane between the control layer and fluidic layer is pushed into the fluidic layer closing the rounded channel in the fluidic layer. There are 8 lines in the control layer to select individual chambers and 5 lines to control the fluid flow from inlets and outlet, see fig. 6.1 for the layout. In total 13 control lines are required to control the operation of one MFBB. The two PDMS layers are bonded together and then mounted on a 60 mm x 30 mm glass slide in accordance with IWA 23:2016 [8]. In the glass slide powder blasted holes are positioned on a standardized grid. These holes provide access to the channels in the control layer, where the bottom of the glass slide provides a flat surface to facilitate a seal. The MFBB has 5 fluidic inlets/outlets which are each connected to individual tubes: a hole is punched through the top PDMS layer to reach the channels in the fluidic layer. The channels in the fluidic layer that connect the inlets to the chamber have matched hydraulic resistance to ensure equal flow rates in the chambers. In the future these external fluid connections could also be interfaced to the fluidic circuit board.

In summary, a 64-chamber building block with 13 control lines to facilitate the individual addressability of the chambers is designed. By adding one more control line several additional chambers could be added to the chip. In theory this is a good option, but in practice this will result in problems, because this will significantly increase the chip footprint. The alignment between the control layer and fluidic layer is critical as it determines the positions where valves are created. Due to the shrinkage of PDMS this becomes more and more difficult as the chips get larger. Furthermore, a smaller chip is less likely to be contaminated by dust resulting in a better yield. Therefore the choice to remain at 64 chambers per MFBB and to use multiple modules is made.

Figure 6.1 - a) Channel routing of the 64-chamber chip with the inlets positioned on the standardized grid, b) Detailed view containing 8 chambers and the control lines needed for individual addressability c) side view showing operation of the PDMS valves.

6.2.2 Fluidic circuit board design

To reduce the amount of tubing needed a fluidic circuit board is developed. This FCB interconnects the three 64-chamber MFBBs to an external interconnect block. The outline dimensions of the FCB are 126.5 mm x 75mm which fits within the standardized microtiter plate [13], see fig. 6.2. In the FCB three sets of 13 valves are integrated, making it possible to enable/disable every single MFBB. In total there are 16 external connections all grouped on a standardized grid and connected to a single connector. Three of these connections control the valves in the FCB to enable/disable a single MFBB. The remaining 13 control lines are connected to the MFBBs, where every MFBB has a set of valves. Each set has 13 individual valves controlled by the same actuation pressure.

To facilitate a seal between the FCB and the MFBB the FCB has pockets that accept the smallest standard size O-ring (AS568-001). The depth of the pocket is designed in such a way that the O-ring is compressed 10%. The pockets together with the flat FCB top and MFBB bottom make a mechanical stop ensuring correct O-ring compression. To align the MFBB to the FCB, dowel pins are sticking out of the surface of the FCB; three for every MFBB. The MFBB can be pushed against the dowel pins to align the inlet holes with the FCB. To ease installation of the clamp, the screws are retained in the FCB. This ensures that they don't fall out during assembly and means there are less small parts which can get lost.

The FCB consists out of 5 thermoplastic layers which are used for valve actuation and fluidic routing. Between the 2nd and 3rd layer, an elastomeric mem-

brane is mounted to be able to open and close the valves. The total thickness of the FCB is 5 mm, providing enough stiffness for clamping the MFBB while still having the possibility to image the chambers with an inverted microscope with limited objective working distance. Furthermore, in the channel routing, care has been taken so there are no features in the FCB under the chambers. On the bottom side the bolts are counter bored so the FCB can lay flat.

Figure 6.2 - FCB layout design.

6.2.3 Auxiliary parts

To mount the MFBB to the FCB a clamp is needed. The clamp facilitates the compression of the O-ring and the mechanical fixation of the block itself. The clamp has three features that prevent breaking of the glass bottom of the MFBB. Correct alignment to the MFBB and FCB is ensured with dowel pins entering holes in the clamp. Line loading on the edge of the glass is prevented by mechanical stops ensuring the bottom of the clamp and the top of the glass are parallel. Furthermore, the screw holes have loose tolerances allowing the clamp to lay flat on the glass, without hanging on the screws. Tightening the screws evenly allows for a uniform pressure distribution over the glass area in contact with the clamp.

The external interconnect block (EIB) makes it possible to connect all 16 tubes at once. It is fixed to the FCB with two screws. The seal between the EIB and

the FCB is made with O-rings. The tubing is connected to the solenoids valves to apply pressure. In the middle of the tubing a set of Luer lock connectors is placed, providing the possibility to fill the tubing and channels with water. This is needed to prevent air passing through the membranes of the PDMS valves into the fluidic layer.

6.2.4 Control system

To control the access of the fluids to the chamber, the valves in the MFBB and the FCB need to be actuated. Solenoids are used to pressurize the control lines, they are controlled through an interface connected to a PC running a Labview program. This way it is possible to run the setup fully automated. It is even possible to cycle through the MFBBs and update their state, similar to the update mechanism of dynamic random access memory.

6.3 Methods and materials

6.3.1 64-chamber microfluidic building block

The 64-chamber MFBB consist of three layers, two Polydimethylsiloxane (RTV 615 PDMS, Permacol BV, The Netherlands) and one glass layer. The PDMS is structured on a mould, SU8 (MicroChem, USA) photoresist is used to create the mould for the control channels as it gives square channels. For the fluidic layer AZ 40 XT (MicroChemicals GmbH, Germany) photoresist is used which gives semi-circular channels after baking it for 90 s at 140 °C. The thin membrane above the control layer is created by spin coating the PDMS (1:20 w/w curing agent to prepolymer) on the mould at 500 rpm for 15 seconds followed by 3000 rpm for 60 seconds resulting in a total thickness of 37 µm. The PDMS (1:7 w/w curing agent to prepolymer) for the fluidic layer is poured on the AZ 40 XT mould until a thickness of around 5 mm is reached. The control layer is pre-cured for 45 minutes at 60 °Celsius. The fluidic layer is pre-cured for 60 minutes at 60 °C. After pre-curing of the fluidic layer, the inlet and outlets holes are punched with a 1mm hole puncher. Both layers are then aligned and pressed together and left to bond and cure overnight at 60 °C. This stack is then plasma bonded to the glass slide. Before bonding, holes are powder blasted in the glass slide on a standardized grid. These holes are also used as alignment marks, thereby avoiding weakening the glass with alignment marks.

6.3.2 Fluidic circuit board design

The fluidic circuit board is ordered commercially from Micronit, The Netherlands. It consists of 5 layers of thermoplastic Polystyrene (PS) and an elastomeric Styrene Ethylene Butylene Styrene (SEBS) membrane. Direct milling is used to create features in the PS layers. The channels controlling the PDMS valves are 300 μm in width and 400 μm in depth. The channels for the pneumatic actuation of the FCB valves are 500 μm in width and 300 μm in depth. The valves in the FCB fit within a footprint of 4 mm². The SEBS membrane is cut with a drag knife on a CNC machine to allow for interconnects to other layers. Subsequently thermal compression bonding is used to bond the layers together. The final step in the process is the milling of the outside contour and drilling the through-holes for the bolts.

6.3.3 Auxiliary parts

The clamp is manufactured from a block of PMMA by direct milling. The external interconnect is also manufactured out of PMMA by direct milling. The diameter of the holes that accept the tubing is 1.4mm. The Tygon tubing (Cole parmer EW-06419-01) with 0.06 inch (1.52mm) OD fits directly in this hole and provides a watertight seal.

6.3.4 Experimental

Three separate experiments are performed on the platform: the first one to determine the correct working of the valves in the FCB, the second to see how long the state of the MFBB is saved after disabling the MFBB with the valves in the FCB and finally the chambers are filled individually with food dye to check for valve leakage in the MFBB and individual building block selection with the FCB.

6.3.4.1 FCB valve characterization

An experiment is performed to determine the opening and closing pressures for the valves in the fluidic circuit board. A Fluigent pressure regulator (MFCSTTM-EZ) is used to apply both the valve gating pressure as well as the fluid pumping pressure. The valve gating pressure is raised from -200 mbar to 1600 mbar in steps of 200 mbar, while the pumping pressure is raised from 0 mbar and 1400 mbar in steps of 200 mbar. The valve gating pressure is increased every 30 seconds; when this is reset to -200 mbar, the pumping pressure is raised. The resulting flow through the valve is recorded with a flow sensor (Fluigent L flow

sensor). Additional tubing is fitted between the reservoir and flow sensor to limit the flow with an open valve. Hydraulic resistance of the tubing is chosen to be around $2\text{mbar}/\mu\text{L}$. A Labview program is developed to control the Fluigent pressure regulator. A Matlab script is used to process the data. To obtain results from the equilibrium states, the first 70% and last 5% of each pressure step is excluded from the mean value.

6.3.4.2 MFBB state saving

To check how well the valves in the FCB sealed, the MFBB is enabled and subsequently the control layer is pressurized to 1400 mbar closing all valves in the MFBB and preventing any flow in the fluidic layer of the MFBB. The MFBB is then “disabled” by closing the valve set in the FCB with a control pressure of 1600 mbar. After this the pressure on the solenoid side of the valve is reduced to 0 mbar. With a leaky valve the pressure in the closed off part of the control layer will drop eventually, allowing flow in the fluidic layer. During the entire experiment a flow sensor is used to measure the flow through the fluidic layer (Fluigent s flow sensor) in the MFBB. The pressure applied to the reservoir connected to the fluidic layer is 150 mbar.

Figure 6.3 - Cross sectional view of valve operation sequence; a) MFBB enabled by opening the valve in the FCB, b) Close the valve in the MFBB by pressurizing the hydraulic actuation layer, c) MFBB disabled by closing the valve in the FCB and d) Solenoid side of the FCB valve is depressurized, waiting state where the pressure drop in the closed off channel will slowly allow flow in fluidic layer. e) leaky state, where the pressure in the control layer has decreased.

6.3.4.3 Individual chamber loading

The complete system behaviour is tested by loading differently coloured food dyes in the chambers of the three MFBB. In sequence the following loading of the MFBBs is performed: A gradient is loaded into the chambers of the MFBB on the left, followed by a gradient loaded into the chambers of the middle chip

and finally a sequential loading of individual chambers in the right chip. The gradient is formed by enabling all three inlets which are connected to food dyes or DI water. The chambers are all differently coloured, due to the structure of channels connecting the inlets to the chambers. The structure contains t-split junctions, making them function as a passive gradient generator, similar to a ‘Christmas tree’ gradient generator [14]. The valves are operated through a Lab-view interface, manually for the gradient loading and automated for the sequential loading. Pressures in the control layer are 0 mbar or 1200 mbar depending on valve actuation status. The pressure in the pneumatic actuation layer in the fluidic circuit board are -200 mbar and 1600 mbar for an open and closed valve respectively.

6.4 Results and discussion

6.4.1 Fabrication results

After fabrication, all part are mounted to check if everything fits. The system looks as shown in fig. 6.4. As can be seen in the figure, three 64-chamber MFBBs are mounted on a single FCB. Connections to the fluidic layer are made from the top of the MFBBs. In the top right corner the external interconnect block (EIB) can be seen, which connects the control lines to the external solenoid valves.

Figure 6.4 - Assembled modular platform, from left to right, three 64-chamber MFBB and an external interconnect block.

6.4.2 Valve characterization

In figure 6.5 the opening and closing behaviour of one of the valves in the FCB

can be seen. For this experiment the MFBB were not mounted. The raw data contained some transient behaviour which doesn't represent the steady state operation of the valve. To obtain steady state data the first 75% and last 5% of a pressure step is removed, resulting in the data shown in figure 6.5.

Figure 6.5 - Resulting flow through a FCB valve for several gating and pumping pressures.

Fig. 6.5 shows that the flow rate is almost fully determined by the hydraulic resistance of the tubing between the reservoir and the valve for the two lowest pneumatic actuation pressures. At a pneumatic actuation pressure of 200 mbar the valve starts to pinch off the flow. For pumping pressures below the pneumatic actuation pressure the valve is completely closed. This can be explained as the membrane area on the actuation side is larger than the area that the fluid pushes against. With equal pressure on both sides there will be a net force keeping the valve closed. For higher actuation pressure this effect becomes more pronounced: at 1400 mbar pumping pressure the valves already fully close at 1200 mbar.

In the corresponding raw measurement data, which is shown in figure 6.6, some

unexpected phenomena were observed. At 200 mbar pneumatic actuation pressure, oscillations of the valve membrane were observed for pumping pressures of 600 and 800 mbar. Out of the 13 tested valves 7 showed these oscillations. These oscillations were observed at different actuation pressures as well as different pumping pressures. Some valves only showed oscillations at a specific actuation pressures and pumping pressures, while others oscillated at multiple pressures. Until now, no explanation for this behaviour has been found. Some back flow was also observed when the pneumatic actuation pressure was increased for a closed valve, this can be explained by the membrane flexing back into the channel (fig. 6.7).

Figure 6.6 - Raw data of flow through a FCB valve for several pneumatic actuation (gray background bars) and pumping pressures.

Figure 6.7 - Flexing of the membrane causing back flow into the channel.

Fig. 6.8 shows the behaviour of several valves at a pumping pressure that is rele-

vant for MFBB valve actuation (1400mbar). Most valves operate in a similar way. Outliers are valves 1, 8 and 13 which need slightly higher pneumatic actuation pressures to fully close.

Figure 6.8 - Opening behaviour of 13 valves at 1400 mbar of pumping pressure.

6.4.3 MFBB state saving

Fig. 6.9 shows the flow through the fluidic layer of the MFBB during the state saving experiment. Around 1 minute after the experiment started, the valves in the MFBB were pressurized and therefore closed. Fig. 6.9 shows that the flow rate is reduced to zero. Subsequently, the MFBB is disabled by closing the valves in the FCB and the solenoid side of the FCB valve is depressurized. The experiment was left to run overnight in this state. Fig. 6.9 has a break in the axis to show the data during the start and end of the experiment. After running for 17 hours and 50 minutes, the valves in the MFBB were still closed. This is longer than will be necessary during normal operation, so the MFBB was enabled and the valves were opened again, resulting in a restored flow in the fluidic layer. The ability of the system to keep the valves closed for such a long time, is likely related to low leakage and the ability to compensate for leakage by having the

membrane of the FCB valve flex in the direction of the closed fluid compartment. Fig. 6.5 supports this hypothesis as a small amount of back flow can be observed when the valve is already fully closed and the pneumatic control pressure is increased. However, this also means that MFBB valves which are open will be partially closed when saving the state of a MFBB.

Figure 6.9 - Flow through the fluidic layer indicating how stable valve states are without actuation.

6.4.4 Individual chamber loading

This concluding experiment shows the working of the total system. In every chamber on the platform a different colour is loaded. In the left and centre MFBBs this is done by creating a gradient between two inlet colours and addressing all chambers at the same time, as individual addressability on these MFBB was faulty due to dust particles between the control lines. In the right MFBB the chambers are individually addressed and loaded with a colour. Fig. 6.10 shows an image sequence taken during the experiment showing the different colours in the MFBBs. The left MFBB is yellow at the start, as the default inlet was connected to a yellow food dye. The centre and right MFBB have the default inlet connected to DI water. As time progresses the three MFBB are subsequently selected. The chambers of the left MFBB are loaded with food dye, followed by the centre MFBB and finally the chambers of the right MFBB are loaded with food dye. The system showed the expected behaviour during the experiment,

indicating all components operated properly and that there was no leakage.

Figure 6.10 - a) Image sequence during loading of three MFBBs. The left and centre MFBB are loaded with a gradient. Individual chambers are loaded sequentially in the right MFBB. b) Detailed view of the chambers in the right MFBB showing the filling of the first two chambers.

6.5 Conclusion

In this chapter a modular system is shown, where high-throughput capabilities are realized by parallelizing three MFBBs with 64-chambers each. The footprint of this MFBB is kept small and is standardized, hereby improving manufacturing yield. Three of these standardized MFBB are interconnected on a FCB to keep the total chamber count high. Minimizing the use of tubing prevents the “tyranny of numbers” problem. One external interconnect block is enough to connect the control lines, which can be used to individually address chambers in the three MFBBs. System functions were characterized or tested. The valves in the FCB close when equal pressures are applied in the control lines and on the fluidic side. The valve states in the MFBB can be saved by closing the corresponding set of valves in the FCB. In the design phase, a state refresh system like DRAM uses was envisioned, but it turned out this was not necessary as the closed states are saved for more than 17 hours. The working of the complete system was shown by loading different combinations of food dye into the individual chambers, which worked as expected. This already provides some answers to the engineering challenges in high-throughput drug screening, however the system still needs to be tested with cell cultures. Currently, the first cell culturing results with human umbilical vein endothelial cell looks promising.

6.6 References

- [1] N.A. Ashammakhi, A. Elzagheid, Organ-on-a-Chip, *J. Craniofac. Surg.* 29 (2018) 823–824. doi:10.1097/SCS.00000000000004604.
- [2] B. Zhang, M. Radisic, Organ-on-a-chip devices advance to market, *Lab Chip*. 17 (2017) 2395–2420. doi:10.1039/C6LC01554A.
- [3] R. Gómez-Sjöberg, A.A. Leyrat, D.M. Pirone, C.S. Chen, S.R. Quake, Versatile, Fully Automated, Microfluidic Cell Culture System, *Anal. Chem.* 79 (2007) 8557–8563. doi:10.1021/ac071311w.
- [4] M. Blazek, T.S. Santisteban, R. Zengerle, M. Meier, Analysis of fast protein phosphorylation kinetics in single cells on a microfluidic chip, *Lab Chip*. 15 (2015) 726–734. doi:10.1039/C4LC00797B.
- [5] S. Woo, L. Ae, S.S. Lee, Shrinkage ratio of PDMS and its alignment method for the wafer level process, (2007). doi:10.1007/s00542-007-0417-y.

- [6] C. Probst, S. Schneider, P. Loskill, High-throughput organ-on-a-chip systems: Current status and remaining challenges, *Curr. Opin. Biomed. Eng.* 6 (2018) 33–41. doi:10.1016/j.cobme.2018.02.004.
- [7] J. Morton, W. Pietenpol, The Technological Impact of Transistors, *Proc. IRE.* 46 (1958) 955–959. doi:10.1109/JRPROC.1958.286834.
- [8] IWA 23:2016 - Interoperability of microfluidic devices -- Guidelines for pitch spacing dimensions and initial device classification, n.d. http://www.iso.org/iso/home/store/catalogue_tc/catalogue_detail.htm?csnumber=70603.
- [9] H. van Heeren, T. Atkins, N. Verplanck, C. Peponnet, P. Hewkin, M. Blom, W. Buesink, J.-E. Bullema, S. Dekker, Design Guideline for Microfluidic Device and Component Interfaces (part 1) ver. 2, 2016. doi:10.13140/RG.2.1.1698.5206.
- [10] H. van Heeren, D. Verhoeven, T. Atkins, A. Tzannis, H. Becker, W. Buesink, P. Chen, Design Guideline for Microfluidic Device and Component Interfaces (Part 2) ver. 1.2, 2016. doi:10.13140/RG.2.1.3318.9364.
- [11] Z. Hua, Y. Xia, O. Srivannavit, J.-M. Rouillard, X. Zhou, X. Gao, E. Gulari, A versatile microreactor platform featuring a chemical-resistant microvalve array for addressable multiplex syntheses and assays, *J. Micromechanics Microengineering.* 16 (2006) 1433–1443. doi:10.1088/0960-1317/16/8/001.
- [12] M.A. Unger, H.-P. Chou, T. Thorsen, A. Scherer, S.R. Quake, Monolithic Microfabricated Valves and Pumps by Multilayer Soft Lithography, *Science* (80-.). 288 (2000) 113–116. doi:10.1126/science.288.5463.113.
- [13] H. van Heeren, Standards for connecting microfluidic devices?, *Lab Chip.* 12 (2012) 1022. doi:10.1039/c2lc20937c.
- [14] A.G.G. Toh, Z P Wang, C. Yang, N.-T. Nguyen, Engineering microfluidic concentration gradient generators for biological applications, (n.d.). doi:10.1007/s10404-013-1236-3.

Chapter 7

Summary and outlook

7.1 Summary

In this thesis the work done in the MFManufacturing project is described. The main goal of this project was to increase the maturity of the microfluidic field. The development of the field, which started around 1990, is described in chapter 1. A comparison is made between the amount of academic papers published field and the amount of patents granted. The Gartner hype cycle is subsequently used to analyse this data. This revealed that the field was not able to live up to its early promises. However, it is now slowly moving a stable plateau by serving a broad business-to-business market with enabling tools for a broad range of custom applications. Unfortunately, there are still a lot of interesting proof of concepts demonstrated by academia which are not commercially available. Lack of standards and tools are one of the possible explanations for the mismatch between the number of publications and commercial available devices.

In chapter 2 this lack of commercialization of microfluidic field was investigated. The focus was put on factors that inhibit the commercialization of microfluidic devices. The following factors were found: their multidisciplinary nature, the need for high volume production, the lack of focus due to their broad applicability, the current patent landscape, the materials used and related manufacturing processes, and the current balance between academia and industry. To overcome some of these inhibitors a standardized modular platform was developed in the MFManufacturing project. With a modular design, large development efforts are better utilized as the modules can be re-used in multiple design. This also helps with the need for high volume production. The standardization efforts focus mainly on the interfacing between the modules to create compatibility between various building blocks. In the standardization, two components are defined: microfluidic building blocks (MFBBs) and fluidic circuit boards (FCBs). The MFBBs contain the microfluidic functions and the FCB connects the MFBBs together. The outside dimensions and inlet/outlet positions are standardized to ensure compatibility.

In Chapter 3, the various standardized dimensions of the MFBBs are discussed, the grid for the interconnections has a preferred pitch of 3 mm and the most common footprint dimension is 15x15 mm, but multiples of this size are also allowed. Some MFBBs and their characteristics are shown, including a pressure sensor, valve and reservoir. This collection of MFBBs is the start of a library containing many microfluidic functions. Furthermore, three different types of FCBs are shown; a polymer one only making fluidic connections, the second one from glass making both fluidic and electrical connections and the last one is a

multi-layered polymer FCB with integrated valves. The materials chosen for the development of these components were chosen in such a fashion that high volume manufacturing is possible. To be able to effectively design new microfluidic systems, a new computer aided design tool was developed in the MFManufacturing project.

In chapter 4, this tool is described, as well as its possibility to simulate a microfluidic system. The tool has the ability to load a library containing MFBBs and contains additional functionalities to make designing according to the standards straightforward. For example: inlets and outlets can be positioned on the standardized grid by use of coordinates. The tool can provide spice simulation to analyse flow and pressure in the complete system. This is different from the current practice in microfluidics, where in-depth simulations are often performed, using methods as finite element models or even molecular dynamics. Unfortunately, the computing power available today doesn't allow to simulate complete systems within an acceptable time frame. The simpler spice simulation provides useful information about the complete system, while having short computation times. It is therefore a useful tool to provide aid during the design process.

To see how well the standardized modular platform performed two systems have been designed according to the standardization. The first system is described in chapter 5 and shows how the use of the platform can move a microfluidic design from a bulky “chip-in-a-lab” to a compact “lab-in-a-chip” solution, resulting in a system that is easier to commercialize. The designed system was an AC coulter counter, which was chosen for its wide applicability, especially as a research tool. The system was tested with several bead solutions containing 11 or 6 μm beads, resulting in detection rates of 99% and 86% for 11 μm and 6 μm beads respectively. Using a solution with a mixture of 11 and 6 μm beads resulted in detection rates of 100% and 39%. The low detection rate of the 6 μm bead is likely caused by the higher flow rates used during this experiment. Video tracking of the beads was used as a reference to obtain the detection rate numbers. With this system it was proven that the standardized modular platform is capable of combining several materials. A glass MFBB was used together with a polymer FCB, where the FCB also contained a pre-amplifier for the electrical signals. By using a custom-made lock-in amplifier the total setup stayed compact, and did not rely on an expensive laboratory grade lock-in amplifier.

The second system makes use of microfluidic large scale integration: in chapter 6 a solution is shown which provides a straightforward way to connect a high

number of valves to their external pressure source. A typical application where this is needed is drug screening in the pharmaceutical industry. The system is based on the standardized modular platform and consists of three 64-chamber MFBB which are interconnected onto a FCB, resulting in a total of 192 chambers. The amount of tubing is minimized due to the combinatorial multiplexing of the control lines in the MFBB, together with chip enabling logic in the FCB. This results in a total of 16 control lines which are connected to the FCB in one external connection block. The valves in the FCB were characterized and the total system behaviour was tested by loading food dye in the chambers. These tests show the system behaves as expected, while using the standardized modular platform resulting in minimal amounts of tubing and high ease of use due to the replaceable MFBBs.

7.2 Conclusion

Overall, inhibiting factors for microfluidic commercialization were analysed. A modular platform was proposed and its accompanying standardization was developed in the MFManufacturing consortium. A limited library containing standardized MFBBs performing basic microfluidic functions was introduced, as well as the FCBs which provide the fluidic and electrical interconnections between the MFBBs. To simplify designing according to the new standard, a computer-aided design tool was introduced, with the possibility to use a MFBB-library and to run microfluidic simulations. The new standardized modular platform was then implemented in two systems: a coulter counter demonstrating a compact “lab-in-a-chip” instead of “chip-in-a-lab” and a microfluidic large scale integration system demonstrating a high number of interconnects with minimal external tubing. However, the success of the standardized modular platform can only be measured in the future by the amount of uptake in the microfluidic field. A standard is only a standard if a broad group in the field is using it.

7.3 Outlook

All work on the standardization part of the modular platform was done in the MFManufacturing consortia, already providing a wide industrial support for the standardization. During the project the ideas were formalized in an ISO-workshop agreement. During this workshop there was also some representation from outside the MFManufacturing consortia, indicating interest from the field. Toward the end of the MFManufacturing project, a workshop on standardization was arranged at the National Institute of Standards and Technology. Around that time, the idea was formed to continue the standardization work in a new

association: “The Microfluidic Association”. Both participants of the MFManufacturing consortia, as well as others active in the microfluidic field joined. Further standardization workshops were organized by the microfluidic association after the end of the MFManufacturing project, indicating an interest from the microfluidic field to continue standardization efforts even though these endeavours are not externally funded. Standardization workshops have been organized at IMEC (November 2017), CEA Leti (March 2018) and MATAS (July 2018) showing there is interest in the standardization of Microfluidic and the work started in the MFManufacturing project is actually actively continued.

Appendix A - Design Guideline for Microfluidic Device and Component Interfaces (part 1)

Editors: Henne van Heeren (enablingMNT), Tim Atkins (Blacktrace/Dolomite), Nicolas Verplanck and Christine Peponnet (CEA-LETI), Peter Hewkin (CfBI), Marko Blom and Wilfred Buesink (Micronit), Jan-Eite Bullema (TNO), Stefan Dekker (University of Twente).

With contributions / suggestions and support from persons from the following organisations: APIX, Axxicon, Bronkhorst, CEA-Leti, CfBI, CMC Microsystems, Corsolution, CytoCentrics, Diagnostics Biosensors, DIBA, Dolomite, enablingMNT, EV Group, EVEON, Fluigent, Fraunhofer IOF, IHP, IMTag, IMTEK, Invenios, IPHT, IVAM, LioniX, Memsmart, Medimetrics, Microfluidic ChipShop, Microfluidic Consortium, Micronit, MinacNed, NIST, Philips, Phoenix, Qmicro, SCHOTT Technical Glass Solutions, Semi, SIMTech Microfluidics Foundry, Skalene, SLAC National Accelerator Lab, Sony DADC, Stanford University, Stiplastics, TNO, University College London, University Twente, and many others.

Comments, suggestions and questions regarding this document can be addressed to: Henne van Heeren, enablingMNT, Netherlands, henne@enablingMNT.com, 0031786300748

This work was supported by the Microfluidic Consortium and the MFManufacturing project, co-funded by grants from the UK, France and the Netherlands and the ENIAC / ECSEL Joint Undertaking.

Version 3.0, June 2017

DOI 10.13140/RG.2.1.1698.5206

Major changes in the White Paper 3.0 versus 2.0:

- Corrected definition edge- / side connector
- Some comments about O-rings

Major changes in the White Paper 2.0 versus 1.0:

Besides correcting typos and adding additional clarifications, the following major changes have been made

- Par. 5.4 Sizes of fluidic ports: Table showing the relation between hole sizes, tissue punch
- size and needle nominal outer diameters.
- Glass wafer thicknesses: transferred to whitepaper part 2
- Side connector: transferred to whitepaper part 2
- Par 8: Exclusion zones around ports and exclusion zones for clamping

In part 2 of this white paper we will address: glass wafer thicknesses, side connector and a route towards smaller chips.

A.1 Introduction

A.1.1 Context

The goal of this document is to facilitate the process of designing new microfluidic sensors, actuators, connectors etc. by providing guidelines for the seamless integration with other microfluidic components and systems. This will overcome the challenge that the process of moving from a research prototype device to a production device takes too long and is too expensive. This is such a barrier for startups in new technology that this phase is sometimes called the “Valley of Death”. This is the case for a wide variety of materials and manufacturing processes. An important part of the challenge is that one often is designing without a clear guideline what the most appropriate method is to integrate it into a system or connect it to other devices. The flip side of the same argument is that potential users are often frustrated when components and systems presented to them are difficult, inappropriate or even impossible to interconnect with or integrate into their systems.

A.1.2 Objectives of this paper

This White Paper is an attempt to improve the situation. It is made available for free to developers and researchers around the world who are contemplating the creation of prototype devices containing microfluidics. Its purpose is to present developers a standard by which they will improve the chances of their device will be accepted by the market / fits to other products.

A.1.3 Positioning of this paper

This paper is “application agnostic” – it is relevant to people working in: Diagnostics, High Throughput Screening, Sample Preparation, Genomics, PCR, Circulating Tumour Cells, Regenerative Medicine, Flow Chemistry, Environmental, Food and Homeland Security Sensing... and beyond!

This paper is also “materials and production technology agnostic” – we recognise that microfluidic devices can be realised in PDMS, PMMA, COC, Polycarbonate, Glass, Silicon, Metal and Paper as different players specialize in / have a preference for different materials. Furthermore this paper is “manufacturing process agnostic” – recognising again that processes can be company specific. Our vision is that newcomers to the microfluidics market – and companies that want to expand their product portfolio – will look at the relevant guidelines and design according to them. The process to create the products based on these

designs will not be described or discussed in this paper. The paper especially addresses topics related to the issue of the microfluidic connections to microfluidic chips or substrates and the integration of microfluidic chips or substrates in components and systems.

Using these design guidelines will be helpful for both user and supplier by ensuring plug and play interconnections.

It is intended that this will be a “living document” updated regularly and the authors are keen on feedback regarding how the document might be improved.

Note: This document does not guarantee IP freedom to operate! There is a complex landscape of patents around microfluidics devices so it is up to you to check whether you need a licence!

The chosen approach concerning the guidelines towards connection/interfacing is to provide the minimum guidelines needed for interoperability, leaving open which materials to be used, what targeted applications and what connections types. We focused on keeping the guidelines simple, understandable by all and implementable by the product manufacturers as well as by the research labs. These guidelines are considered as a first essential step but certainly not an end point.

A.2 Definitions around chips and connectors

Connection the microfluidics on a chip or substrate to the outer world is less straightforward than many may think. Often this involves connecting manually channel by channel; a laborious activity and often leading to malfunction. This can be improved by using standardized (multiport) connectors. To enable these, agreements have to be made about the dimensions of the chips and the positions of the ports. As this industry lacks a common language even the discussion itself is complicated.

In order to clarify this, a few definitions will be given below. Also the area where our guidelines apply will be described.

We have considered two parts particularly important for interoperability: the chip and the connector.

The chip is a flat microfluidic device. Important are its format and the position

of the fluidic inlets and outlets. Our guidelines will specify at least:

- The chip format
- The inlet /outlet port location

The connector is defined by two sides; the side connected to the chip and the other side that is left open and can be connected to a tube, an instrument, a fluidic circuit board, another chip, a sensor, etc...

Although one can easily represent a chip, it is much less true for the connector which is important to consider in a very broad way. Indeed in establishing these guidelines, we considered as connector not only typical connectors such as those sold by Dolomite, Micronit and others, but also the possibility to use other types of assembly methods such as adhesives (double face tape, glue...), O-rings with a clamping system or even the often used mini-Luers.

Not defining completely the connectors but only the side connected to the chip gives a huge freedom to operate, independently of the chip material or the chip to chip assembly method. However, defining only the geometry (port location, and foot print) is still a great achievement since it enables interoperability.

By defining only the chip geometry we avoid all the chip to chip, chip to fluidic circuit board, chip to outer world discussions, simplifying the problem to the chip and to the chip side of the connector. This simplicity enables a large number of users and manufacturers to consider using these guidelines.

In order to better clarify chip topology we have agreed on the following terminologies represented in Figure A.1:

- Top or Bottom connections (ToB connections)
- Side connections

Figure A.1 - Schematics showing definitions of top, side and edge (left); top connection (middle) and side connection (right).

Figure A.2 defines the nomenclature of the top and bottom chip sides as well as the left right upper and lower sides. It should be noted that the choice for defining the top and bottom sides is based on manufacturing practices. The person that is using such a chip under a microscope might have an opposite view!

Figure A.2 - Schematics showing top, bottom and sides of a chip

In some cases the using of the terms top and bottom side of the chip might cause confusion; for instance when such a chip is mounted on a microfluidic circuit board. In such cases, it is advised to use the terms interfacing side (where the ports are) and clamping area (the other side).

A.3 Pro forma standard chip sizes and interconnections

Although many different chip sizes are being used and can be used for microfluidics, for several reasons it might be advisable to adhere to certain chip sizes that are commonly used and supported by the supply chain.

The analytical industry is using microtiter plates with standardized dimensions. (See: ANSI SLAS 1-2004 (R2012), formerly recognized as ANSI/SBS 1-2004). Based on this specification microfluidic chips are offered which have the same outer dimensions. When this chip size is used for microfluidics, the microfluidic connections are mostly miniLuers, placed on the borders of the chip with a pitch of 4.5 mm (or multiples of 4.5 mm) according to the positions of the outer wells of the standard layout. See Figure A.3:

Figure A.4 - Layout of microscope slide commonly used in microfluidics with miniLuer interconnect positions. Although often used, the format above shows several disadvantages, especially when one wants to use multiport connectors, connectors with less dead volume or when one wants to use smaller chips. Therefore, the following paragraphs will present a more advanced set of standard configurations, containing optimized standard chip dimensions and positions of microfluidic ports.

A.4 Standard guidelines for axes and reference point

The objective of this part of the design guide is to provide a coherent system of reference axes used to describe positions on the chips. The convention is based on the chip manufacturing view point, not on the user who might take another view. We only address rectangular and square chips. The reason for this, lies in the objective of this paper, providing guidelines for microfluidic interconnections and integration. Circular disks are not discussed as they seldom need microfluidic connections and are generally used as standalone devices, i.e. seldom integrated.

A.4.1 XY axes for microfluidic chips

General comment: the XY axes are chosen in such a way that for the description of a certain point only positive numbers need to be used.

Rectangular chips

The naming of the chip defines the position of the XY axes. For instance a 15*30 mm² chip has the X axes along the 15 mm side. The chip is then posi-

tioned with the X axis pointing from left to right. The Y axis is on the left of the viewer. The reference point is then on the top left of the chip (see next Figure).

Figure A.5 - 15*30 mm² chip with axes and reference point.

A 30*15 mm² chip has the X axis along the 30 mm side. The Y axis is again on the left and the reference point on the top left corner. (See Figure A.6.)

Figure A.6 - 30*15 mm² chip with axes and reference point.

As a preference one should chose the naming (and with that the X axis) in such a way that (most of) the microfluidic connections are on the side of the X axis.

Square chips

For square chips, the positioning of the XY axis is more arbitrary, but again the preference is that (most of) the connections are near the X axis. If that would

lead to two different options due to asymmetric placement of the microfluidic ports, one should choose the one with most of the ports near the reference point (See Figure A.7).

Figure A.7 - Preferred positioning of XY axes and reference point for square chips.

A.4.2 Chip reference point

The reference point is the point where the two sides, named X and Y axes, cross.

Figure A.8 - Position of reference point determined by the two axes.

When a chip has rounded corners, as it is often the case with injection moulding, the crossing planes of the two sides of the chip, will be used as the reference point (Figure A.9).

Figure A.9 - The position of the reference point at the crossing planes (A and B) of the two sides of the chip in presence of rounded corners.

If needed, in the case of symmetrical chips for example, the position of the reference point might be clearly marked by a marker in the vicinity of the reference point. If this is the case, a preferred option would be to locate identification markers on the left side of the chip in an index area. This is analogous to identifying or alignment markers in electronics (sometimes also called fiducials).

Several Identifying Markers can be used:

- Painted or printed markers are recommended when a simple orientation check of the chip is needed.
- A corner cut or notch can be considered if this is needed for the assembly process.

A.5 Microfluidic port

This section describes the available options for positioning the microfluidic ports on a chip, its size and the coding of the port position. This is particularly of relevance for those that want to design chips and ToB connectors that are truly interchangeable. These positioning rules are also used for the definition of the side connector standard. But that is not all, these also provide guidelines for those that have an interest to connect microfluidic sensors and actuators to microfluidic circuit boards. Finally it had an influence on the early discussions about very small microfluidic chips and their connections.

A.5.1 Port pitches

This section describes the available options for positioning the microfluidic ports on a chip. Of all geometrical dimensions, the port pitch, i.e. the distance between the centres of two ports is perhaps the most important parameter. It was decided that all the proposed layouts are to be based on a 1.5 mm grid. Not all the holes are necessarily present on the chip or connector, but all the hole positions are fixed. This 1.5 mm grid enables several configurations. The hole positioning is always established from the reference point, in order to avoid cumulative drifts. A 1.5 mm pitch was chosen for the following reasons:

- Below 1.5 mm, according to manufacturers, it is at this moment not possible to have leakage free fluidic connections using the currently available multi-port connection technologies.
- Microtiterplates are a well-established standard for tissue culture, cell-based assays, high throughput/content screening applications, etc. In addition, there are well established peripheral equipment suppliers for microtiterplate based liquid handling, processing and detection. The well positions in microtiterplates are compatible with the 1.5 mm grid. Therefore 1.5 mm is also a good number to accommodate:
 - MiniLuers (widely used in microfluidics) have a 4.5 mm pitch.
 - Microtiter plate well spacing for reservoirs filling by a multi pipette for example.

Figure A.10 - Top view of ToB connections showing the position of the row of hole at a distance of centre to centre of 1.5 mm from each other on the X axis.

There is a preference for a 3.0 mm port pitch ($n=2$). In the microfluidic field, 3 mm spacing is considered as state of the art for spacing between ports. In the near future chips and connectors with 1.5 mm pitches will likely become available.

A.5.2 Nominal distance of the top or bottom fluidic port from the sides of the chip/substrate

Due to restrictions in manufacturing technology it was decided that the centre of a top or bottom port should always be at a distance of at least 3 mm from all sides.

This 3 mm distance was adopted after discussion with injection moulding manufacturers, assuring that such a distance from the edge ensured a robustness when using injection technologies.

Following this restriction, the position of the first allowed hole/fluidic port position near the reference point is now defined as being at position (3 mm, 3 mm). (see Figure A.11).

Figure A.11 - Top view of ToB connections showing the position of the first hole at a distance of 3 mm from each side of the top left corner of the chip.

A.5.3 Distance between two rows

Distance between two rows is a multiple of 1.5 mm ($p \cdot 1.5$ mm) from centre to centre on the y axis (Figure A.12). Not all the rows are necessarily present on the chip or connector, but row positions are fixed. This 1.5 mm grid enables several configurations. The row positioning is always established from the reference point, in order to avoid cumulative drifts. The same reasoning was applied to the Y axis as the one used for the X axis. There is a preference for a 3.0 mm row pitch ($p=2$).

Figure A.12 - Top view of ToB connections showing the position of the rows at a distance of centre to centre of 1.5 mm from each other on the y axis.

A.5.4 Sizes of fluidic ports

Controlling the diameter for fluidic ports are important for leak free connections, reduction of dead volume and flow resistance. As diameter sizes are very much dependent on the fabrication technology it is very difficult to standardize them in total. However, minimum and maximum recommended diameters (d) of the port on the surface of the chip are:

- 1.5 mm pitch : $0.4 < d < 0.7$ mm
- 3.0 mm pitch : $0.4 < d < 2.0$ mm
- 4.5 mm pitch : $0.4 < d < 3.5$ mm

Indeed, depending on the needed pressure and flow rate, diameters may need to vary in order to control pressure loss and dead volumes. However microfluidic chip and connector manufacturers considered that a distance of 1 mm between holes is required to have a good microfluidic connection. This therefore provided the upper limit of the range. An exception was made for the 1.5 mm pitch for which 0.7 mm maximum was required in order to provide a sufficient port diameter.

For PDMS chips, university researchers widely use tissue punch or syringe needle to fabricate fluidic access hole. In addition, for PMMA/PDMS chip, many university researchers glue syringe needles on the chip inlet/outlet as fluidic access interface with tubing. Both tissue punch or syringe needle have their own size standard. The defined hole sizes are compatible with the tissue punch sizes, as shown in the following table:

Table A.1 - Relation between hole sizes, tissue punch size and needle nominal outer diameters

Pitch (mm)	Hole size (mm)	Tissue punch size (mm)	Needle Nominal outer diameter (gauges)
1.5	$0.4 < d < 0.7$	0.5	27-17 gauges
3	$0.4 < d < 2.0$	0.5, 1	27-12 gauges
4.5	$0.4 < d < 3.5$	0.5, 1, 1.5, 2	27-8 gauges

A.5.5 Fluidic port nomenclature

Fluidic port nomenclature was proposed to simplify fluidic chips design, guidelines and instructions. We have adopted the same convention as the microtiter plate format used to identify wells: numbers for columns and letters for rows.

The first possible grid position in the left corner, closest to the reference point, would be A1, the next one on the X axis with a distance of 1.5 mm will be A2. Numbers increase from left to right and letters from upper to lower edge (Figure A.13).

The fluidic port grid follows the standard pitch definition of 1.5 mm and can unambiguously identify port locations on a chip. The chess board type notation for fluidic ports helps to prevent mistakes between fluidic ports and electrical interconnect in a chip design where both types of interconnects are used. The generic grid is 1.5 mm pitch but the 3 mm pitch corresponds to the preferred configuration.

Figure A.13 - Top view of ToB connections showing fluidic port, nomenclature based on a 1.5 mm grid.

As a final remark it should be noted, that this universal system for port positioning still needs in each case clarification about the character of each port (incoming, outgoing fluids etc.) or even existence of the port: not all positions might be used for microfluidic interconnections.

A.5.6 Something about O-rings

The diversity in O-ring dimensions is high and due to the size constraints microfluidic engineers have no option but chose the most optimal O-ring for their application. If possible, however, we recommend to use metric dimensions. Unfortunately, ISO-norms for O-rings do not cover yet the small dimensions needed by this community. It is recommended to use commercially available ones

that follow or extend the dimension systematics of the larger dimensions.

Table A.2 - Recommended O ring dimensions

Inner Diameter	Cross section
1	1
1.5	1
2	1
2.5	1
1	1.5
1.5	1.5
2	1.5
1	2
2	2
Etc.	Etc.

A.6 Summary table of all interconnect guideline dimensions and tolerances regarding port pitches, chip thicknesses and port dimensions

The relevant guidelines for ToB connectors are summarized in the table below:

Table A.3 - Key parameters for top interconnection standardization and tolerances.

Parameters	Nominal value	Minimal value	Maximal value	Tolerance
Reference point : Left chip corner				0 mm
Distance of the first hole from the reference point (3 mm, 3mm) (corner edge to hole centre)	(3 mm, 3 mm)			+/- 0.15 mm
Minimal distance of any hole from any side of the chip		3 mm		
Distance between holes or port pitch (centre to centre)		1.5 mm		+/- 0.15 mm
Rows are parallel to the chip's x axis at a distance from ref. point of n*1.5		1.5 mm		+/- 0.15 mm
Port diameter for 1.5 mm grid		0.4 mm	0.7 mm	
Port diameter for 3 mm grid		0.4 mm	2.0 mm	
Port diameter for 4.5 mm grid		0.4 mm	3.5 mm	
Tight tolerance of outer chip dimension (desired)				+0.05 / - 0.15 mm
Lower tolerance of outer chip dimension (when tight tolerance not achievable)				+/- 0.3 mm

A.7 Standard guide lines for chip formats

Due to the diversity in the market, standardisation of chip formats is a difficult topic, but after analysing the situation and discussions with major players, we were able to propose here some guidelines that fit as best as possible to the demand. We added to those standard formats the position of the microfluidic ports according to the standards we developed before (see section 4).

A.7.1 Outer chip dimensions in general

The outer chip dimensions are important for standardization. Chip formats are described in the next section. However it is important to fix the desired tolerances, while allowing for less accurate manufacturing processes. We have defined an asymmetrical tolerance for limiting oversize chips which will not fit in holders and connectors:

- Maximum / desired oversize is (+ 0.05 mm)
- Maximum undersize is (- 0.15 mm)
- Preferred undersize is (- 0.05 mm)

A.7.2 Microscope slide format standards

The official microscope slide standard allows all sizes in length between 76 and 75 mm and in width 26 and 25 mm. This will not work for affordable and reliable connectors. The two most commonly used dimensions are 75.6 x 25.4 and 75 x 25. We have chosen the slide format that fits best to the grid of 1.5 mm, which is therefore the 25.0 x 75.0 mm slide format.

In order to have symmetrical connectors, we have modified the first hole location for this format: the first hole position will be at 3.5 mm¹⁰ from the long edge and 3.0 mm from the short edge.

Microscope slide X1:

Figure A.14 - 75*25 mm microscope slide with ports having 3 mm pitches (blue); the microtiter plate well positions in a 9 mm grid are shown for reference (in orange)

Microscope slide X2:

Some users have a double microscope slide format, in this case 50.0 x 75.0 mm is the standard chip format. The first hole position will be 4 mm from the long edge and 3.0 mm from the short edge.

Figure A.15 - 75*50 mm microscope slide with ports having 3 mm pitches (blue); the microtiter plate well positions in a 9 mm grid are shown for reference (in orange)

A.7.3 Credit card format

The credit card format with its well-established manufacturing production lines is often used in the industry and may be important for providers of microfluidic devices. For this reason, we have included this format which is 85.60×53.9812 mm. To make it better compatible with the 3 mm pitch preference, we propose the following “credit card” format: 84 mm x 54 mm (see Figure A.16).

Figure A.16 - Top view of credit card chip format showing in blue the standard fluidic ports for one row and one column.

A.7.4 Smaller chip format standards

Although, as shown above, there are standard chip formats for the larger chip sizes, no such standard exists yet for smaller chips. That is an unfortunately situation; there is much interest to go to smaller chip sizes. For the moment we propose to use footprints that are a multiple of 15 mm in the X and Y directions. This size has been chosen because an investigation by one of the MFManufacturing partners showed that this is a format used by several companies. It is also identical to the standard coverslips: 15*15; 30*30 and 60*60.

Furthermore, it is compatible with the 1.5 mm pitch and can accommodate a wide range of applications. That is not the end of the discussion; already there are voices that vote for chip sizes smaller than 15*15 mm.

A.8 Exclusion zones

To be able to achieve a leak free connection and a mechanical strong fixation, a sealing area on the interfacing side (where the ports are) and an area reserved for clamping the chips are needed.

A.8.1 Exclusion zone for the interfacing area (where the microfluidic ports are).

In order to achieve leak free microfluidic connection between a chip and a connector it is advised to define a 6×6 mm² square with centre port in the middle of that square. Prohibited in this zone are: structures that create obstructions on the surface and structures that create holes, dents etc. in the surface (except the portholes itself of course). (see next figure)

Figure A.17 - Exclusion zones around portholes; example 15*15 mm chip

A.8.2 Exclusion zone for clamping of the chip

If a sensor or actuator chip is attached to a circuit board with clamping designers should take care that a strip of 1 mm wide at the opposite side of the chip is reserved for clamping purposes. This area should be free of obstructing structures. (see next figure)

Figure A.18 - Clamping zone for 15*15 mm² chip

Although a clamping zone of 1 mm wide is sufficient for chips with sizes of 15*15 mm² or smaller, for larger chips wider clamping zone might be needed. Larger clamping zones may also be needed for applications where pressures up to 30 bars are being used.

A.9 Sensor / Actuator building blocks

In many cases it is easier to position a sensor direct onto another component or device without using tubes for interconnections. For instance placing them on a microfluidic circuit board analogue to placing a transistor on an electronic circuit board. This part of the guidelines defines the geometries required to ensure plug and play interconnections and interoperability in such situations. Mechanical fixture can be done for instance by gluing or clamping. We advise here generic design rules for the templates of the most common building blocks (15x15 and 15x30).

We identify three types of connections: fluidic connections, pneumatic and electrical connections.

A.9.1 15x15 Microfluidic Building Blocks

Figure A.19 - Top view 15x15 Microfluidic Building Block with multiple ports.

Figure A.20 - Top view of 15x15 Microfluidic Building Block with ports centred on short edges.

Figure A.21 - Top view of 15x15 Microfluidic Building Block with a single centred port in D4 position.

A.9.2 30x15 and 15x30 Microfluidic Building Blocks

Figure A.22 - Top view of a 30x15 mm building block with ports on the long side.

Figure A.23 - Top view of a 15x30 mm building block with ports on the short side.

Figure A.24 - Top view of a 15x30 mm building block with centred ports.

A.9.3 15x45 Microfluidic Building Blocks

Figure A.25 - 15x45 Microfluidic Building Block with multiple ports on the short side.

Figure A.26 - 15x45 Microfluidic Building Block with centred ports.

A.9.4 Sensor / Actuator Microfluidic Building Block with one inlet and one outlet

Figure A.27 - Sensor block layout with fluidic and electrical interconnections (1)

Figure A.28 - Sensor block layout with fluidic and pneumatic interconnections (2)

A.9.5 Example of the use of standardized sensor / actuator interfaces

The next figure shows an example where companies have made use of the standardized sensor / actuator layout (dimensions and port pitches). The components on the microfluidic circuit board (they are from several suppliers), all adhere to the proposed standards discussed above. The valves and sensors (light blue), the interface block (red) and the actuator (yellow), all are 15 *15 mm2 blocks. The microfluidic ports connecting those parts to the microfluidic circuit board (not visible) are all on the grid positions described before.

Figure A.29 - Example of the use of sensor / actuator standard to create a complete functional tool (courtesy EVEON)

A.10 Standard guidelines for operational conditions / application classes

This part is still under discussion and is provided here as an example of how we will proceed rather than a final version of operational condition limits. We came to this point by an iterative process using surveys to check our initial assumptions. Temperature and pressure were chosen as key elements for the operating conditions while they are closely linked to the applications and have important consequences in terms of manufacturing chips or connectors. Depending on the conditions temperature or pressure, material choice will be impacted as well as the design.

The first survey provided a coarse overview of the field. The proposed temperature and pressure ranges for the second survey were chosen based on the following considerations:

- The temperature range 4 - 50 °C was chosen while this range is used by many suppliers of off the shelf pumps.
- The temperature range 4 - 100 °C is chosen to cover also applications using polymerase chain reaction (PCR)
- On advice of some suppliers, who felt that the difference between 50 and 100 °C was too much, 75 °C was chosen as a upper limit too.
- 2 And 7 bar are maximum pressures that are quite commonly used by suppliers to specify their products. The other limits are based on the outcome of the first survey. The second and third survey provided more information about the distribution of the user over the classes. (see Figure A.30)

Figure 8.30 - Distribution of the users of microfluidics over the classes (from a survey).

Based on this outcome we created a first proposal for operating classes:

Table A.4 - Proposal for application classes.

Classes	Maximum pressure (bar)	Maximum Temperature (°C)	Minimum temperature (°C)
PT 2/50	2	50	4
PT 2/75	2	75	4
PT 2/100	2	100	4
PT 7/50	7	20	4
PT 7/100	7	100	4
PT 30/50	30	50	4

Table A.4 presents only a starting point in relation to classification. Undoubtedly a more comprehensive classification scheme is envisaged, once other distinctive features like for instance flow and media used will be taken into account. It is therefore likely that other classes and sub-classes will be introduced into the scheme of things, which will be explored in follow up surveys. For example, we will explore if this classification system can be extended to cover different media used, thus taking into account if the device is designed for chemicals or biologicals. In addition, the classification system will need to take into account special devices, such as those that operate at higher pressures/temperatures than those listed in the table. For example, the classification system will need to be extended to take into account special cases such as HPLC-Chip technology, where pressures are significantly higher than those listed.

A.11 And Finally

As said, this White Paper is not a final document; it is just a reflection of the first discussions about microfluidic standards. Several new topics will be addressed in part 2 and 3 of this white paper, addressing chip thicknesses, side connectors and further miniaturization. Experienced engineers will find many other details to specify. For instance those interested in shorter times to market and higher reliability will stress the need for industry wide accepted validation tests etc. etc.. Therefore we (and hopefully you too) will regard this as a living document. We are interested in your feedback and involvement to improve it!

We are grateful to the ENIAC /ECSEL project MFManufacturing and the Microfluidic Consortium that have supported this work. And thanks to many, many engineers and researchers who actively participated in the many discussions leading to this document.

Samenvatting

Het werk dat is beschreven in dit proefschrift is gedaan in het kader van het MFManufacturing project. Het hoofddoel was het verhogen van de volwassenheid van het microfluidische vakgebied. De ontwikkelingen in dit vakgebied, dat rond 1990 startte, zijn in hoofdstuk 1 beschreven. Er is een vergelijking gemaakt tussen de hoeveelheid gepubliceerde papers in de academische wereld en de hoeveelheid toegekende patenten. Vervolgens is de zo genoemde “Gartner hype cycle” gebuikt om deze data te analyseren. Dit liet zien dat het vak gebied niet instaat was om zijn initiële beloftes waar te maken. Niettemin, heeft het vakgebied een brede reikwijdte als ondersteunende technologie in verschillende applicaties. Hierdoor ontwikkeld het vakgebied zich langzaam tot een stabiele business-to-business markt. Helaas zijn er nog steeds veel academische gedomestreeerde concepten die het uiteindelijk niet redden als een commercieel product.

Het gebrek aan commercialisering in het microfluidische vakgebied is onderzocht in hoofdstuk 2. Dit hoofdstuk concentreert zich op factoren die de commercialisatie van microfluidische apparaten verhindert. De volgende factoren spelen een rol: hun multidisciplinaire aard, de eis van grote productie volumes, gebrek aan focus door brede toepasbaarheid, het huidige patent landschap, de gebruikte materialen en bijbehorende fabricage methoden en de huidige onbalans tussen academie en industrie. Om deze verhinderde factoren te overwinnen is er een gestandaardiseerd modulaair platform ontwikkeld in het MFManufacturing project. Door het gebruik van modulariteit kunnen grote ontwikkel investeringen beter verdeeld worden, doordat de modules ingezet kunnen worden in meerdere ontwerpen. Dit is ook voordelig voor de groottes van de productie volumes. De standaardisatie richt zich vooral op de raakvlakken tussen de modules, door het creëren van compatibiliteit tussen de verschillende bouw blokken. In de standaard zijn twee soorten componenten gedefinieerd: een microfluidische bouw blok (MFBB) en een fluidisch circuit bord (FCB). Het MFBB bevat de microfluidische functionaliteit en het FCB verbindt alle MFBB met elkaar. De buiten afmetingen en de posities van de ingangen/uitgangen zijn gestandaardiseerd om compatibiliteit te garanderen.

In hoofdstuk 3 worden de verschillende gestandaardiseerde afmetingen besproken. 3mm is de geprefereerde afstand in het raster voor de interconnecties en 15x15 mm is de meest voorkomende footprint afmeting, voor grotere chips zijn veelvoud van deze afmeting ook toegestaan. Er is een opsomming aanwezig

van een aantal MFBBs en hun karakteristieken. Deze opsomming bevat onder meer een druk sensor, een klep en een reservoir. Deze collectie van MFBBs is een start van een bibliotheek welke een groot aantal microfluidische functies bevat. Verder worden er 3 verschillende types FCB getoond; een polymeer FCB voor fluidische verbindingen, een glazen variant die ook in elektrische verbindingen voorziet en een complexe polymeer variant met geïntegreerd kleppen. De gebruikte materialen zijn zo gekozen dat hoge fabricatie volume mogelijk is. Om effectief gebruikt te maken van de beschreven bibliotheek is nieuwe software ontwikkeld in het MFManufacturing project.

In hoofdstuk 4 is deze software beschreven, samen met de mogelijkheid om simulaties van een microfluidisch systeem uit te voeren. De software heeft de mogelijkheid om MFBB uit een bibliotheek te laden en bevat aanvullende functionaliteit om een ontwerp te maken welke voldoet aan de standaard. Een voorbeeld: in- en uitgangen kunnen op een standaard raster geplaatst worden door middel van coördinaten systeem. De software biedt de mogelijkheid om de druk en stroming in het gehele systeem te analyseren door middel van spice simulaties. Dit verschilt van de huidige methode, welke vaak gebruikt wordt voor het simuleren van microfluidische systemen. Vaak worden diepgaande simulatie uitgevoerd, gebaseerd op eindige elementen methodes of zelfs op moleculaire dynamiek. Er is helaas vandaag de dag niet genoeg rekenkracht om dit soort simulatie uit te voeren voor een compleet systeem binnen een acceptabele tijd. De simpelere spice simulaties bieden nuttige informatie over de werking van het complete systeem binnen een korte tijd. Hierdoor is het een nuttige gereedschap dat ondersteuning biedt tijdens het ontwerp proces.

Om te zien hoe goed het gestandaardiseerde modulaire platform presteert zijn er twee systemen ontworpen welke voldoen aan de standaardisatie. Het eerste systeem is beschreven in hoofdstuk 5 en laat zien hoe het modulaire platform bijdraagt aan de overgang van een omvangrijk “chip-in-a-lab” systeem naar een compact “lab-in-a-chip” systeem. Het ontworpen systeem is een wisselstroom coulter counter welke een breed inzet gebied kent, vooral in het onderzoek. Het systeem werd met verschillende oplossingen getest. De oplossingen bevatten 6 μm en 11 μm beads, waar van respectievelijk 86% en 99% gedetecteerd werden. De oplossing met een mix van 6 μm en 11 μm resulteerde respectievelijk in 39% en 100% detectie. Het lage detectie percentage voor de 6 μm beads in dit experiment werd waarschijnlijk veroorzaakt door een hoger vloeistof snelheid. De beads werden gevolgd in de video data om een referentie te verkrijgen voor het berekenen van het aantal gedetecteerd beads. Met dit systeem is bewezen dat het

gestandaardiseerd modulaire platform in staat is om verschillende materialen in een systeem te combineren. Een glazen MFBB werd samen met een polymeer FCB gebruikt. Het FCB bevat ook een voorversterker voor de elektrische signalen. Door gebruikt te maken van een specifiek ontworpen lock-in versterker kon de totale opstelling compact blijven. Bovendien was het gebruik van een dure laboratoriumkwaliteit lock-in versterker niet meer vereist.

Het tweede systeem maakt gebruik van “microfluidische grote schaal integratie”: in hoofdstuk 6 is een systeem beschreven dat het makkelijk maakt om een groot aantal kleppen met de bijbehorende druk bron te verbinden. Een toepassing waar dit benodigd is het testen van grote hoeveelheden medicijnen in de farmaceutische industrie. Het systeem beschreven in hoofdstuk 6 is ook gebaseerd op het gestandaardiseerde modulaire platform en bestaat uit drie 64-kamer MFBB welke met elkaar zijn verbonden op een FCB, wat resulteert in een totaal van 192 kamer. De hoeveelheid externe slangen is geminimaliseerd door het gebruik van combinatorische multiplexing van de controle kanalen in de MFBB en de chip selectie logica in de FCB. Dit heeft geresulteerd in een totaal van 16 controle lijnen welke verbonden worden met de FCB in één extern verbindingsblok. De kleppen in de FCB zijn gekarakteriseerd en het gedrag van het totale systeem is getest door het laden van verschillende kleurstoffen in de individuele kamers. Deze test toonde aan dat het systeem naar behoren werkt, terwijl het gestandaardiseerde modulaire platform resulteert in een minimaal aantal slangen en een groot gebruiksgemak door de uitwisselbare MFBBs.

De toekomst ziet er ook positief uit voor de standaardisatie van het microfluidische vakgebied. Het werk dat gestart is binnen het MFManufacturing project wordt doorgezet door een nieuwe associatie. De “Microfluidic Association” bestaat uit leden van het MFManufacturing project en andere geïnteresseerden uit het vakgebied. Er zijn inmiddels vier workshops georganiseerd om het standaardisatie werk voor te zetten. Dit geeft goede hoop aangezien deze workshops volledig door het vakgebied zelf bekostigt worden. Het werk van de “Microfluidic Association” zal verdere standaardisatie naar het vakgebied brengen.

Scientific output

Peer-reviewed journal articles

S. Dekker, W. Buesink, M. Blom, M. Alessio, N. Verplanck, M. Hihoud, C. Dehan, W. César, A. Le Nel, A. van den Berg, M. Odijk, Standardized and modular microfluidic platform for fast Lab on Chip system development, *Sensors Actuators B Chem.* 272 (2018), 468–478.

In press: **S. Dekker**, P.K. Isgor, T. Feijten, L.I. Segerink, M. Odijk, From chip-in-a-lab to lab-on-a-chip : a portable coulter counter using a modular platform, *Nature Microsystems and Nanoengineering*, doi: 110.1038/s41378-018-0034-1.

In preparation: A. Vollertsen, **S. Dekker**, B. Wesselink, R. Haverkate, J. Bomer, H. S. Rho, R. J. Boom, M. Skolimowski, M. Blom, A. D. van der Meer, R. Passier, A. van den Berg, and M. Odijk, Standardized PARallelization of multichamber Chips, the SPARC platform.

Conference contributions

S. Dekker and M. Odijk, Towards a Standardized Interconnect Solution, a Fluidic Circuit Board, Lab-on-a-Chip & Microfluidics (poster presentation), March 15-16, 2015, Berlin, Germany.

S. Dekker, A. van den Berg, M. Odijk, Standardized and modular microfluidic platform for fast lab on chip system development, *microTAS 2017* (poster presentation), October 22-26, 2017, Savannah, USA. ISSN(electronic): 1556-5904.

A. Vollertsen, **S. Dekker**, B. Wesselink, R. Haverkate, J. Bomer, H.S. Rho, R. Jan, Standardized , Modular Parallelization Platform for Microfluidic Large-Scale Integration Cell Culturing Chips, *microTAS 2018* (poster presentation), November 11-15, 2018, Kaohsiung, Taiwan, ISSN(electronic): 1556-5904.

Other

H. van Heeren, T. Atkins, N. Verplanck, C. Peponnet, P. Hewkin, M. Blom, W. Buesink, J.-E. Bullema, **S. Dekker**, Design Guideline for Microfluidic Device and Component Interfaces (part 1) ver. 2, 2016. doi:10.13140/RG.2.1.1698.5206.

ISO international workshop agreement 23:2016 - Interoperability of microfluidic devices -- Guidelines for pitch spacing dimensions and initial device classification.

S. Dekker and M. Odijk, A flow sensor as a modular building block, Second Workshop on Standards for Microfluidics (Oral presentation), October 9-10, Leuven, Belgium.

F. van den Brink, **S. Dekker**, S. Roelofs and M. Odijk, Electrical Engineering meets Chemistry, De Vonk (periodical of the E.T.S.V. scintilla , University of Twente), 33(2), 10-15

Funding and contributions

Funding

The work presented in this thesis is mainly done in relation with MFManufacturing project. This project was funded by ECSEL Joint Undertaking (JU), a public-private partnership focusing on nanoelectronics that brings together ENIAC Member/Associated States, the European Commission, and AENEAS (an association representing European R&D actors in this field). The project is known under proposal number 621275-2, with the proposal acronym MFManufacturing.

The work presented in chapter 6 was partly funded by the VESCEL ERC Advanced Grant to A. van den Berg (Grant no. 669768)

Contributions

The MFManufacturing project consisted of a large consortium with the following members. Micronit, Dolomite, University of Twente, Nederlandse organisatie voor toegepast-natuurwetenschappelijk onderzoek (TNO), Commissariat à l'énergie atomique et aux énergies alternatives (CEA), National Physical Laboratory management Limited, Axxicon, Philips, Medimetrics, Apix, STI plastics, Tronics microsystems, Eveon, PMB, Visco, Phoenix, Fluigent and enablingMNT. All these members contributed to the process of defining standards for modular microfluidic design, as shown throughout this thesis.

Chapter 1 & 2

Chapter 1 and 2 written by Stefan Dekker with feedback from Laura Folkertsma-Hendriks and Mathieu Odijk.

Chapter 3

Several MFBB are shown in Chapter 3 to show an initial library of standardized parts. The differential pressure sensor, reservoir and simple polymer-based FCB were developed and manufactured by Stefan Dekker, the valve MFBB and reaction chamber were developed and manufactured by CEA-LETI. The pump MFBB was developed and manufactured by Eveon. The complex polymer-based

FCB and glass-based FCB together with the clamping solutions were developed and manufactured by Micronit. The characterization of the blocks was subsequently performed by the same companies, or the author of this thesis. This chapter was written by Stefan Dekker with feedback from and Mathieu Odijk.

Chapter 4

The design software shown was written by Phoenix and adapted from its original purpose as a design tool for photonics. The spice models for simulating microfluidic structures were developed by TNO and Stefan Dekker. The system was subsequently tested to see what its capabilities were by Stefan Dekker. This chapter was written by Stefan Dekker with feedback from and Mathieu Odijk.

Chapter 5

The design and fabrication of the system shown in chapter 5 was done by master student Tobias Feijten, in close collaboration with his supervisor Stefan Dekker. Initial experiments were performed by Stefan Dekker, while Pelin Isgor took over to perform the final experiments. The data processing was mainly done by Stefan Dekker. The initial draft of this chapter was written by Stefan Dekker with subsequent improvements suggested by Pelin Isgor. Final feedback was received from both Loes Segerink and Mathieu Odijk.

Chapter 6

The idea of the system shown was born by the need of many culture chamber, Anke Vollertsen already had developed microfluidic chips containing tens of culture chamber. Pre-master student Rob Haverkate made an initial effort in making these chips compatible with the standardized platform and extend the number of chambers per chip to 64-chambers. The information gained during this project was used by Anke Vollertsen and Stefan Dekker to subsequently make a new design. The new design holds 3 times 64-chambers. The fabrication of the FCB system for this system was outsourced to Micronit. The 64-chamber chips were fabricated by Anke Vollertsen. This chapter was written by Stefan Dekker with feedback from Anke Vollertsen and Mathieu Odijk.

Dankwoord

Hier het laatste hoofdstuk van mijn proefschrift, voor velen het eerste deel dat gelezen word. Ik wil dit dankwoord graag gebruiken om de vele personen waarmee ik heb samengewerkt te bedanken. Zonder jullie was dit proefschrift niet mogelijk geweest.

Ik wil graag beginnen met Mathieu Odijk. Tijdens mijn master opdracht had jij een open positie, deze had een praktische insteek en zal vol met samenwerkingen met het bedrijfsleven. Dit leek mij wel wat. Niet veel later was ik begonnen aan mijn PhD avontuur. Ik had altijd het gevoel dat je het volle vertrouwen in mij had, zeker in de begin periode. Terwijl jij werk in Amerika verrichte, had ik mijn eerste MFManufacturing meeting waar ik de Universiteit Twente vertegenwoordigde, deze kan ik mij nog goed herinneren. Dit was een goede mogelijkheid om alle partners in het project te leren kennen. Onze twee wekelijks meeting waren altijd prettig, door onze discussies bracht je me altijd op nieuwe ideeën. Ook wanneer het niet zo soepel liep, bijvoorbeeld tijdens het schrijven, was er altijd jou helpende hand, dit stelde ik altijd zeer op prijs.

Ten tweede wil ik Albert van den Berg bedanken voor de mogelijkheid om in zijn vakgroep te werken, waar de sfeer altijd heel prettig is. Ik heb altijd ook erg genoten van het jaarlijkse mountainbiken en wil jou en Trudy bedanken voor het beschikbaar stellen van jullie tuin voor de daarop volgende BBQ. Tijdens deze BBQ had ik altijd het gevoel alsof ik op vakantie was.

Even terug in de tijd, aangezien mijn carrière bij BIOS al langer loop dat alleen mijn PhD. Mijn eerste aanraking met de BIOS vakgroep was via Wouter Olthuis. Hij heeft mij fantastisch ondersteund bij het samen stellen van mijn vakken pakket tijdens mijn master. Hierop volgden twee opdrachten bij BIOS. De ontwikkeling van FDTs coater als individueel project in mijn pre-master, waarbij ik veel ondersteuning en begeleiding heb gekregen van Jan van Nieuwkastele. De kennis die ik hier heb opgedaan over mechanische systemen kwam tijdens mijn PhD zeker van pas. Hierop volgde mijn master opdracht, het sorteren van beads gebaseerd op hun grootte. Van mijn begeleider Bjorn Wagenaar heb ik veel geleerd, zowel praktisch in het lab als tijdens het schrijven van mijn verslag. Tijdens de twee wekelijks meeting met Bjorn Wagenaar en Loes Segerink ontstonden altijd goed discussies over het werk dat al gedaan was en wat er nog moet gebeuren. Ik wil al deze personen bedanken voor hun samenwerking en het voorbereiden van mij op mijn PhD periode.

Gedurende mijn PhD periode heb ik natuurlijk ook met veel personen samengewerkt. Te beginnen met de partners binnen het project. Ik wil Marko Blom van Micronit als project coördinator bedanken voor het draaiend houden van het project waardoor ik met de volgende mensen samen kon werken. Ik zou Maurice van der Beek en Peter Bouma willen bedanken voor onze prettige samenwerking en de mogelijkheid om bij jullie (Philips, Eindhoven) langs te komen om het een en ander te bespreken en jullie labs te bezoeken. Dit bezoek werd vaak gecombineerd met een bezoek aan Medimetrics, hier wil ik Ventzeslav Iordanov en Klaas Kerkhof bedanken voor de prettige samenwerking. Ook wil ik Wilfred Buesink bedanken voor de mogelijkheid om zo af en toe bij Micronit binnen te stappen, soms om wat materiaal te halen andere keren om te overleggen. Dit stelde ik altijd er op prijs.

Andere betrokkenen bij het project zag ik vaak tijdens de halfjaarlijks besprekingen, waar ik altijd nieuwe ideeën kreeg door de gevoerd discussies. Ik wil de volgende personen graag specifiek bedanken voor het werk dat we samengedaan hebben: Nicolas Verplanck (CEA Leti), Majid Hihoud (Eveon), William César (Fluigent), Anne Le Nel (Fluigent). Verder wil ik alle betrokkenen bij het MFManufacturing project bedanken voor hun bijdrage en het daarmee mogelijk maken van het werk wat ik de afgelopen 4 jaar heb gedaan. Als laatste wil ik Henne van Heeren van EnablingMNT bedanken voor zijn interesse in mijn werk en het delen van zijn uitgebreide netwerk.

De ideeën die tijdens de project meeting werden opgedaan werden verder uitgewerkt op de universiteit. Ik wil mijn kantoor genoten bedanken voor de prettige werk omgeving en het altijd openstaan om vragen te beantwoorden of een korte discussie te voeren. Adithya Sridhar, Burcu Gümüşcü en Floris van de Brink wil ik bedanken voor het verwelkomen van mij in hun kantoor toen ik begon met mijn PhD. Adithya werd tijdens mijn PhD periode afgelost door Jeroen Vollenbroek, wie ik wil bedanken voor de prettige samenwerking en het altijd beschikbaar zijn om wat te prutsen aan een elektronische schakeling. Burcu werd afgelost door Corentin Tregouet, waarmee de gesprekken in het Nederlands steeds uitgebreider werden.

Tijdens mijn PhD periode bij BIOS heb ik 3 studenten gehad welke mij geholpen hebben met mijn onderzoek. Ik wil Yi Wang bedanken voor zijn hulp met de bonding testen. Tobias Feijten wil ik bedanken voor het starten van de ontwikkeling en fabricage van de coulter counter, wat tot een mooi hoofdstuk heeft geleid in dit proefschrift. Als laatste wil ik Rob Haverkate bedanken voor

het voorwerk dat hij gedaan heeft om het 192-kamer systeem te realiseren, wat ook als hoofdstuk in dit proefschrift staat.

Verder wil ik Pelin Isgor bedanken voor haar hulp met het voorzetten van het werk dat Tobias gestart is. Onze lange discussies hebben geleid tot een beter publicatie dan dat ik alleen had kunnen realiseren. Ook wil ik Anke Vollertsen bedanken voor de goede samenwerking in het verder ontwikkelen van het systeem waar Rob een start mee maakte. Ook wil ik Maciej Skolimowski en Robert Jan Boom van Micronit bedanken voor fabricatie van het FCB voor dit systeem. Deze samenwerkingen hebben geresulteerd in een snelle realisatie van een goed werkend systeem, hierdoor was het mogelijk om dit resultaat op te nemen in mijn proefschrift.

Ik wil ook mijn huisgenoten Hai The Le en Jean-Baptiste Blondé bedanken voor de gezellige ritjes van en naar de Universiteit. Jullie waren altijd een motivatie om samen met jullie naar de UT te gaan.

Verder wil ik iedereen binnen BIOS bedanken voor de altijd prettige sfeer en de leuke activiteiten die we samen ondernomen hebben. Ik wil Paul ter Brake, Lennart de Vreede, Martijn Tibbe en Hugo Albers bedanken voor het organiseren van de zeil weekenden, ik vond het altijd leuk om met een groep biotes te gaan zeilen. Gedurende mijn periode bij bios mocht ik twee werk weken meemaken, een keer naar Engeland en een keer naar Zwitserland. Wesley van den Beld, Floris van den Brink, Burcu Gümüşcü, Yawar Abbas en Paul ter Brake, Anne Leferink, Miguel Solsona, Anke Vollertsen bedankt voor het organiseren van deze onvergetelijke weken. Miguel Solsona, Allison Bidulock, Hoa Tran, Juen Wang, Vasillis Papadimitriou, Hermine Knol – de Vries, Laura Folkertsma, Martijn Tibbe, Lennart de Vrede, Tanja Hamacher, Lisette Knippenborg, Tina Hoekema, Christina Tiflidis, Hai The Le, Jorien Berentsen, Josh Loessberg-Zahl, Paul ter Brake, Loes Segerink, Jan Eikel, Marinke van der Helm, Wesley van den Beld, Bjorn Wagenaar, Rogier Schoeman, Yawar Abbas, Hugo Albers, Pelin Isgor, Anke Vollertsen, Julia Mikhal, Jasper Lozeman, Eshter Tanumihardja, Elsbeth Bossink en Pascal Furher en natuurlijk de grote hoeveelheid studenten bedankt voor het creëren van de prettige sfeer binnen BIOS

Jan van Nieuw Kastele wil ik bedanken voor zijn hulp met het opzetten van de freesmachine. Zonder deze machine was een groot deel van mijn onderzoek niet mogelijk geweest. Ik wil Hans de Boer bedanken voor het meedenken over hoe bepaalde onderdelen gerealiseerd kunnen worden en zijn advies op mechanisch

gebied. Ik heb hier veel van geleerd. Ik vond het altijd leuk als andere mensen geïnteresseerd waren in deze fabricage techniek. Binnen bios wil ik Jeroen Vol-lenbroek, Elsbeth Bossink, Christina Tiflidis en Martijn Tibbe bedanken voor de goed samenwerken en het realiseren van leuke ontwerpen met de freesmachine. Vanuit andere vakgroepen was er ook interesse, ik wil Aisen de sa Vivas, Wesley Buijsman en Brigitte Bruijns bedanken voor de samenwerking welke hebben geresulteerd in chips, chip houders of mallen om chip te maken. Het was altijd een leuke uitdaging om de verschillende ontwerpen te realiseren.

Als laatste wil ik mijn ouders bedanken. Ik ben altijd welkom bij jullie in Ande-ren. Ik kan hier altijd goed tot rust komen en er is altijd tijd voor een gesprek, het maakt niet uit of het even niet lekker loopt of dat ik een leuke ervaring wil delen. Pap en Mam bedankt voor het altijd welkom zijn bij jullie.

Als afsluiter, iedereen bedankt voor deze goed PhD periode.

ISBN: 978-90-365-4611-9