Designing Microfluidic Control Components

Wouter van der Wijngaart

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Wouter van der Wijngaart

Microsystem Technology
Department of Signals, Sensors and Systems (S3)
Royal Institute of Technology (KTH)

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The two front pictures were found on http://www.szgdocent.org and show two water insects.

The top left picture shows a pond skater or water strider (Gerridae). Pond skaters skate on the water surface by distributing their weight over long thin stilts-like legs. These legs have waxy hairs to avoid breaking the water surface tension. Skaters groom themselves regularly to make sure their legs remain waterproof, combing out dirt from the hairs and spreading a thin coating of oil over the hairs. Some bits of their legs, however, penetrate the water surface tension, so they can get a “grip” and push off. They appear to have only four legs because their front legs are very short. They paddle with the middle pair of legs, steer with the hind legs and use the short front legs to attack and hold prey. They move rapidly, up to 35-125 cm/second, and can also jump into the air, some up to 50 cm.

The bottom right picture shows a backswimmer (Notonectidae). Like other freshwater insects, backswimmers exploit water surface tension. But unlike most, they live UNDER the water surface, hanging from the surface, swimming upside down with their long flattened fringed legs. Their bellies are brown to match the mud to hide from predators above the water, and their backs are a speckled black and white to blend with the water surface and hide from underwater predators.
Abstract

This work is a microfluidic engineering study. Its overall goal is to create new components and applications through the downscaling of fluidics and to solve technological bottlenecks in this field for better performance of existing solutions.

Three specific application areas within microfluidics are studied. A first area is liquid diffuser micropumps. The work focuses on solving one of the main bottlenecks for such micropumps, namely reliability. A new pump design with enhanced bubble tolerance is presented, featuring as the first pump of its kind bidirectional pumping of both gas and liquid with the same device. Moreover, a deeper insight of the flow behaviour in the micropump chamber is given. The device can pump a wide variety of liquids that are of interest in biotechnical applications, and pumping of beads and living cells was shown.

The second area this work focuses on is handling and control of on-chip nanolitre liquid volumes. Specifically, a flow-through filter device for bead handling was designed and fabricated. A novel fluidic interface design, a new fabrication method for hydrophobic control elements and a novel fluidic control method are presented, allowing spatial and temporal control of parallel on-chip processes with a minimal device interface. The fabricated devices were successfully used for SNP (single nucleotide polymorphism) analysis with pyrosequencing techniques.

The third part of the work is in the area of micro gas handling devices. A novel pneumatic-to-mechanical-vibration energy converter concept, intended for wireless energy supply, is presented. The other work performed in the micro gas handling field focuses on cost efficient design of microvalves for large pneumatic energy control. A novel electrostatic large-stroke valve actuator for the control of large pneumatic pressures is presented and investigated. The combination of pressure balancing and flexible electrode structures form the key to the actuator performance. Measurements on test structures confirm the modelled actuator behaviour and predict a significant performance increase compared to earlier electrostatic valve actuators. Another part of the work uses analytic flow predictions and FEA (finite element analysis) to study the gas flow through microvalve nozzles. A novel design, consisting of multiple boss elements and orifices, is proposed. The design allows optimisation of the valve's flow capacity and reduces the actuator stroke that is required.

Wouter van der Wijngaart,

Microsystem Technology, Department of Signals, Sensors and Systems (S3), Royal Institute of Technology (KTH), SE-10044 Stockholm, Sweden

Keywords: microfluidic, micropump, microTAS, microvalve, diffuser, bubble, reliability, piezoelectric, electrostatic, pneumatic, actuation, energy conversion, hydrophobic valve, DNA, SNP, pyrosequencing, miniaturisation00, fluidic interface, tube coupler, filter-chamber, beads, parasitic charging, pressure balancing, microflow, CFD (computational fluid dynamics), FEA (finite element analysis).
“Microfluidics is half of the MEMS field.”

(Discussible statement from a passionate researcher)
A chilly morning

Later our friend regretted it wasn’t one of the first, or last, mornings of the week. Then, at least part of the trauma to follow could have been blamed on the excesses of the preceding evening.

He woke up hungry and freezing. His breath was that of an asthmatic’s. The reluctance to leave bed was greater than usual - almost as if his pyjamas had been glued to the sheets, but the leap to the soft and nearby carpet made him feel light as a bird and actually gave him a bruise when he accidentally hit the ceiling.

In the bathroom the water of the basin offered him a remarkably concave surface and it was with great difficulty he managed to get his face into it, just to find himself very similar to those Apollo guys, looking into the mirror above with all the water evenly distributed around his head.

A few minutes later, exhausted from the drying procedure, and now really starving, he entered the kitchen where his better half, struggling to separate the pages of the morning paper, saluted him. By his opinion this work seemed pretty meaningless since the letters were all blurred anyway. Half a dozen eggs went down the saucepan and having boiled furiously for just a few seconds, his wife (wise from her own experience) picked them up with a spoon, claiming they were ready to eat.

To his immense surprise they were…

(From Lilliputian Reflections by Greger Thornell)
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Papers

1. The first self-priming and bi-directional valve-less diffuser micropump for both liquid and gas
   Wouter van der Wijngaart, H. Andersson, P. Enoksson, K. Norén and G. Stemme
   IEEE Proceedings of the thirteenth annual conference on Micro Electro Mechanical Systems, Myazaki,

2. A valve-less diffuser micropump for microfluidic analytical systems
   Helene Andersson, W. van der Wijngaart, P. Nilsson, P. Enoksson and G. Stemme

3. Micromachined flow-through filter-chamber for chemical reactions on beads
   Helene Andersson, W. van der Wijngaart, P. Enoksson, G. Stemme

4. Hydrophobic valves of plasma deposited octafluorocyclobutane in DRIE channels
   Helene Andersson, W. van der Wijngaart, P. Griss, F. Niklaus and G. Stemme

5. Micromachined filter-chamber array with passive valves for biochemical assays on beads
   Helene Andersson, W. van der Wijngaart and G. Stemme

6. A micro pneumatic to vibration energy converter concept
   Wouter van der Wijngaart, A. Berrier and G. Stemme

7. A high-stroke, high-pressure electrostatic actuator for valve applications
   Wouter van der Wijngaart, H. Ask, P. Enoksson and G. Stemme

8. A study of orifice-controlled flow for microvalve design optimisation
   Wouter van der Wijngaart, A. Olsson and G. Stemme
   Submitted.

Additional work

9. SNP analysis by allele-specific extension in a micromachined filter chamber
   Afshin Ahmadian, A. Russom, H. Andersson, M. Uhlén, G. Stemme and P. Nilsson
The contribution of Wouter van der Wijngaart to the different publications:

1. All design\(^1\), all fabrication, all experiments, and major part of the writing.
2. All design, major part of fabrication, part of the experiments, and part of the writing.
3. Major part of the design, part of the experiments, part of the writing.
4. Part of the design, part of the writing.
5. Major part of the design, part of the experiments, part of the writing.
6. Major part of the design, part of the fabrication, part of the experiments, major part of the writing.
7. Major part of the design, part of the fabrication, part of the experiments, major part of the writing.
8. All design, major part of the simulations, major part of the writing.
9. Wouter's name was erroneously left out of this paper, although he provided the major part of the device design.

---

\(^1\) The diffuser micropump principle has been studied extensively earlier [11], however all work that was reported in this paper was performed by the author.
1. Objectives and overview

This work is a microfluidic engineering study. Its overall goal is to create new components and applications through the downscaling of fluidics and to solve technological bottlenecks in this field for better performance of existing solutions. Specific application areas studied are reliability of liquid diffuser micropumps, handling and control of on-chip nanolitre liquid volumes, cost efficient design of microvalves for large pneumatic energy control and a pneumatic pressure driven energy converter for wireless power supply applications.

Because of the broad scope of the work presented in this thesis, the introduction will not try to overview other work and solutions where this is not necessary for the understanding of the novel designs in focus.

Chapter 2 gives a general introduction to the field of microfluidics. Chapter 3 describes some of the typical characteristics of and problem areas within microfluidics. This chapter is intended to both provide a broader background and place this thesis in relation to some general design issues within microfluidics. Chapters 4, 5 and 6 give a broader background to design issues within the three specific areas of research: diffuser micropumps, microfluidic analysis tools for biotechnical and chemical applications, and gas flow microvalves. The concluding chapter summarises the work presented in this thesis. Further, the published papers are summarised and the appendix contains a popular scientific article overviewing the work on the analysis tool for biotechnical and chemical applications.

I wish you, my reader, happy reading.
2. Size matters\(^{(2)}\)

This chapter gives the reader a short introduction to the *Microworld* and the world of microfluidics.

### 2.1. The Microworld

The world that we, humans, live in and that we perceive in daily life is in the scale of a couple of kilometres (a landscape) down to a part of a millimetre (sand grains). I will call this world the *Macroworld*. The world with a physical scale between a couple of millimetres (chip size) and the sub-micron scale (the feature size of electronic components) I will call the *Microworld*. It is the “daily world” for small insects and bacteria.

The *Microworld* differs from the one most people experience, as is illustrated in the short story titled “A chilly morning” in the first pages of this work. The reason for this is that different forces dominate this scale. This is clearly illustrated by the anatomy and behaviour of insects. From an engineering point of view, they can be seen as extremely complicated microsystems that are self-powered, contain multiple sensory input components and have autonomous transport mechanisms for movement on land, in the underground, in water or in the air.

![Figure 1](image.jpg)

*Figure 1. Two insects from the class Hymenoptera. Insects can be seen as autonomous automotive self-powered multifunctional systems with specialised sensory input components.*

### 2.2. Microengineering

Microengineers started entering the *Microworld* only a quarter of a century ago. They typically try to utilise the fascinating possibilities that the *Microworld* offers in new applications in the *Macroworld*. Therefore, microengineers must transform a *Macroworld* task into a *Microworld* task where it can be carried out in a controlled manner and thereafter transferred back into the *Macroworld* where it can be applied. The challenge for microsystem engineers lays thus partly in interfacing between the *Macroworld* and the *Microworld*, partly in controlling of *Microworld* phenomena. This is illustrated in Figure 2.
Figure 2. The Macroworld task needs to be transformed into a Microworld task, where it can be processed in a controlled manner.

Generally, microsystem technology is applicable in every situation where using the benefits of the physical scaling laws or of microfabrication technology creates a more beneficial result in terms of performance or cost.

2.3. Microfluidic devices

The devices and device components this work describes are designed to control either gas or liquid (or both) in the Microworld. From a design perspective, microfluidic components can be divided into three groups, based on their functionality. The first group includes devices that control the (pneumatic) properties of an off-chip fluid medium. A second group of devices controls on-chip fluid samples for sample investigation purposes. Such devices are mainly used in biotechnical and medical applications. A last group consists of interfacing microdevices whose main task is to deliver small controlled amounts of fluid to an off-chip environment. All three groups contain devices featuring on-chip actuation, active components, and devices with off-chip actuation, passive components. Some examples are given in Table 1. This classification is not an attempt by the author to create a novel strict classification tool for microfluidic devices, but rather to group devices that show similar design criteria and typically face similar design problems.
<table>
<thead>
<tr>
<th>Functionality</th>
<th>Active components</th>
<th>Passive components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Off-chip fluid control</td>
<td>• Stand-alone micropumps</td>
<td>• Microfilters</td>
</tr>
<tr>
<td></td>
<td>• Active microvalves</td>
<td>• Passive microvalves</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Micromixers</td>
</tr>
<tr>
<td>On-chip fluid control</td>
<td>• Electrophoresis devices</td>
<td>• Micro reaction chambers</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microfluidic interfacing</td>
<td>• Inkjet print heads</td>
<td>• Transdermal drug delivery needles</td>
</tr>
<tr>
<td></td>
<td>• Microdispensers</td>
<td>• Fume nozzles</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Mass spectrometer needles</td>
</tr>
</tbody>
</table>

*Table 1. Some examples of microfluidic control components, classified according to their functionality.*
3. Characteristics of microfluidics

This chapter is intended to provide the reader with a broader background and to place this work in relation to some general design issues within microfluidics.

3.1. Surface

One of the direct consequences of miniaturisation is that the surface to volume ratio increases linear with decreasing feature size. The relatively large surfaces in the *Microworld* result in increased physical interaction between the different material phases. This gives some interesting challenges and a range of possibilities. Some of the effects that determine the essence of microfluidics are given in Table 2. The physical effects and fabrication features forming the basis for the designs studied in this work are discussed in more detail in subsections 3.2 to 3.4.

<table>
<thead>
<tr>
<th>Phase interface</th>
<th>Phenomenon</th>
<th>Microfluidic design characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solid-liquid</td>
<td>Friction</td>
<td>Laminar flow predominates</td>
</tr>
<tr>
<td></td>
<td>Adhesion</td>
<td>Capillary phenomena and hydrophobic control</td>
</tr>
<tr>
<td>Liquid-gas</td>
<td>Surface tension</td>
<td>Gas bubble problems</td>
</tr>
<tr>
<td></td>
<td>Evaporation</td>
<td>Liquid systems must be closed</td>
</tr>
<tr>
<td>Solid-solid</td>
<td>Friction</td>
<td>Diaphragms as mechano-fluidic transducers</td>
</tr>
<tr>
<td>Solid-gas</td>
<td>Friction</td>
<td>Gas flow losses</td>
</tr>
<tr>
<td>All</td>
<td>Heat transfer</td>
<td>Rapid heating and quenching</td>
</tr>
</tbody>
</table>

*Table 2. Most of the phenomena and challenges occurring in microfluidics are the result of an increased surface to volume ratio.*

Section 3.5 in this chapter addresses fluidic interfacing, which is a fabrication or functional challenge rather than a pure small-scale phenomenon.

3.2. Surface tension: wet dream or nightmare?

Small volumes have a relatively large surface. One of the most important physical effects influencing liquid control on the microscale is therefore surface tension. The surface tension of gas bubbles in liquid is a first example of a typical microscale phenomenon that challenges the microengineer, and is discussed in more detail in a first subsection. A second subsection describes the use of hydrophobic patches as passive valve elements.
3.2.1. Bubble paranoia

Because surface tension increasingly dominates on a microscale, gas bubbles in liquid must be either controlled or totally avoided. Microliquid components that do not make use of surface tension phenomena often show reliability problems due to the “parasitic” occurrence of gas bubbles in the device. Typical devices suffering from this are micropumps and microvalves driven by mechanically moving parts. During the design of liquid handling components, the occurrence of gases must be accounted for because surface tension effects, if they occur, can efficiently destroy the device functionality. Some of the nasty things bubbles do include blocking channels [1], buffering dynamic actuation pressure, or simply changing the effective internal geometry of liquid recipients, as described in chapter 4.

However unwanted, small gas bubbles enter the component, e.g. when they are mixed in the liquid, because of out-gassing of the liquid or due to cavitation inside the component. Once bubbles are created, surface tension causes them to stick to the inner surface of the device where they remain. The smaller the device features, the larger the problem.

![Diagram of bubble problem and solutions]

Figure 3. Two strategies can be followed to solve bubble problems.

One strategy when addressing this issue, as indicated in Figure 3, is to avoid bubble formation at any cost. This typically involves a flow duct design that does not trap gas pockets during priming, degassing liquids before introducing them into the device, using liquids that are less prone to bubble formation and limiting the system actuation to regimes in which no cavitation occurs. A different strategy was deployed in the research on reliable diffuser micropumps described in this work. Its focus lays on bubble-tolerant actuation rather than on bubble prevention. This is necessary if one cannot accept a narrowly specified type of actuation regime or liquid handled.

3.2.2. May the force be with you

A next surface tension related phenomenon is capillarity. The capillary force is a combination of the surface tension effect and the adhesion between liquid and solid. It occurs as a line force at the gas-solid-liquid interface and can be expressed as $F_{\text{cap}} = L \cdot \gamma \cdot \cos(\theta_c)$, where $L$ is the length of the interface line, $\gamma$ the surface tension coefficient and $\theta_c$ the wetting angle. For engineering purposes, one often recalculates the capillary force on the solid-liquid-gas interface to an equivalent pressure $P_{\text{cap}}$ on
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the liquid-gas interface surface as \( P_{\text{cap}} = \frac{F_{\text{cap}}}{A_{\text{cs}}} \), where \( A_{\text{cs}} \) is the cross-sectional area of the liquid duct and \( F_{\text{cap}} \) the line force on the edge of the liquid-gas interface. Active control of the capillary force has been reported, where the dependency of the liquid contact angle on the electrostatic field strength \( \theta_c(E) \) [2], or the dependency of the surface tension on the temperature \( \gamma(T) \) [3] is used. Engineering the inner surface coating [4] and/or the cross-sectional geometry of the fluid duct [5] allows passive surface tension control.

The laws of scaling are exemplified in Figure 4, where the gravitational force, the pressure force and the capillary force on a water droplet in a tube are compared. The calculation of the forces assumes a circular tube cross-section and a water droplet size defined by the tube diameter and a length of 10 tube diameters. To express a net surface tension force on the droplet, one part of the inner tube surface is assumed to have no affinity to water (contact angle \( \theta_c=90^\circ \)) while the other side has a contact angle \( \theta_c=0^\circ \) (perfectly hydrophilic). The pressure used for the comparison is 10kPa. Figure 4 shows that for channel diameters in the microscale, the capillary force on the liquid sample is dominant. Controlling liquid in a microsystem thus requires controlling the capillary forces that are present.

![Figure 4. Illustration of how the laws of scaling influence the forces on a water droplet in a tube: the surface tension (dashed), pneumatic pressure (dash-dotted), and gravitation-induced forces (dotted) on a liquid droplet are plotted versus the tube diameter.](image)

Passive microfluidic components that use capillary forces for liquid control are hydrophobic patches [4], described in paper 4. They consist of a hydrophobic material that is selectively coated on the inner surface of a hydrophilic liquid duct. The function of such an element is illustrated in Figure 5. Liquid fills the duct capillary until the solid-liquid-gas interface reaches the hydrophobic patch. To make the liquid front cross the hydrophobic patch, a pressure drop over the liquid-air interface larger than the threshold pressure \( P_{\text{th}} \) of the patch is required. The threshold pressure is the capillary pressure of the hydrophobic patch and can thus be approximated as \( P_{\text{th}} = \frac{\gamma_{\text{la}}}{A_{\text{cs}}} \int_{S} \cos(\theta_c(s)) \, ds \), where \( S \) is the three-phase interface line. The equation is written in its more general form to allow taking into account situations where the interface line runs over materials with different contact angles [6]. The pneumatic
pressure over the liquid sample thus controls its position. This configuration therefore functions as a passive valve element and allows liquid control both in space and in time. The exact positioning of the patch on the chip during fabrication performs spatial control. Temporal control is performed through the combination of the pneumatic control signal and the definition of the threshold pressure during fabrication. The latter is determined by the choice of the surface coating and the channel geometry. Small channels will exhibit large values of capillary suction pressure for hydrophilic inner surfaces, but will exhibit large threshold pressures if the inner coating is hydrophobic.

This type of passive valve element is attractive for on-chip fluid control because of its ease of fabrication (paper 4), low chip area consumption and relative insensitivity to clogging.

3.3. Planarity and diaphragms

In micropump and microvalve design, diaphragms are often used as a basic building block. This is not a coincidence, as is explained in this subsection. Figure 6 gives a schematic design analysis of mechanically actuated microfluidic devices. Because of the high surface to mass ratio of microstructures, friction is a dominant phenomenon in Microsystems. Moreover, the typically high operating frequency of microstructures makes sliding elements prone to failure. Therefore, moving parts of microstructures should not slide over another surface and must therefore be either flexible or freehanging.

Microfluidic devices that use a mechanical actuator element to generate or transduce a pressure often require the moving actuator part to be leak-tight. Therefore, the actuator typically consists of a
Diaphragm. Examples of such structures are the pump diaphragm of the diffuser micropump, presented in papers 1 and 2, and the pressure balanced electrostatic valve actuator, presented in paper 8. The inherent stiffness of diaphragms and the fracture risk of very thin structures mean that a rather large diaphragm area is required for a certain stroke-length. It can also be noticed that many commonly used actuators, e.g. piezoelectric, thermal, magnetic or shape memory alloy (SMA) actuators, are bimorph structures that preferably move perpendicular to their largest dimension. Moreover, out-of-plane structuring of materials that differ from the bulk (silicon) material is not straightforward. Therefore, such actuators are often out-of-plane moving planar structures.

However, a large pressurised diaphragm area requires the control of large pneumatic forces. Fabrication cost and the limitations of the work actuators can deliver make it therefore necessary to carefully design and optimise microfluidic diaphragm actuators. Subsection 4.2.2 describes how the diaphragm is optimised for the diffuser micropump, section 6.2 describes the diaphragm design of a pressure-balanced electrostatic valve actuator and section 6.3 outlines a method to reduce the requirements on valve actuator stroke-length.

The above analysis also explains why out-of-plane movement is a design requirement for the energy converter presented in paper 6. Out-of-plane vibrations are simply easier to transfer to other microdevices and the possibility for future incorporation of other components with the energy converter is made easier.
3.4. Gas flow losses

The small dimensions of microstructures severely limit their gas flow capacity and give rise to large frictional gas flow losses. Microvalve designs for controlling a large gas flow are described in chapter 6. This section describes an application where the inherent flow resistance of small-scale systems is used as a functional property.

3.4.1. Flow resistance as a component requirement

Pressurised gas has a large energy density and is widely available in industrial complexes. A study on a converter concept that uses this energy supply for measurement and control purposes is described in paper 6. Measurement and control components require a relatively low supply power however. The converter should therefore be designed for limited pneumatic energy consumption. This makes a microsystem with its inherent flow resistance a viable candidate as a converter element. The studied converter design is depicted in Figure 7.

![Figure 7. Schematic view of the converter design.](image)

The gas through the system runs from the high-pressure supply, through a supply nozzle to form a shallow gas bearing underneath a suspended disc and finally escape into the surrounding atmosphere. The suspended disc vibrates on the gas cushion. In this manner, the pneumatic energy is converted into a mechanical vibration of the disc. The shallow gap between the substrate and the disc makes this system inherently flow resistive and thus attractive from a converter design perspective. Paper 6 describes a model of the gas bearing that explains some basic phenomena of the system behaviour. A discussion on the energy conversion phenomena and measurement results from a test structure are also incorporated in the paper.
3.5. Interfacing to microfluidic systems

This section focuses on interfacing between the *Macroworld* and the *Microworld*, a main challenge for microengineers.

### 3.5.1. General aspects

Both fluidic interfacing and control interfacing is necessary to and from microdevices. The interface to the microsystem is the place where the *Macroworld* meets the *Microworld*. Depending on the specific application, fluids must be introduced to or removed from the microsystem. In addition, external control of the fluidic actuation is often needed or measurement results need to be collected from the system.

Interfacing creates specific problems. First, the fluidic interface does typically not contribute to the functionality provided by the device miniaturisation. On the contrary, it constitutes a dead volume that counteracts the benefits from the on-chip size reduction. Moreover, interfacing requires relatively large on-chip footprints and therefore, the interface, rather than the on-chip functionality, often defines the component size and the feasible degree of miniaturisation and parallelisation. Finally, the device interface is typically not batch fabricated and thus expensive. Therefore, it is important to minimise the system interfacing.

Below, a novel tube connection interface is described in the first subsection and a non-contact liquid interface in the next.

### 3.5.2. Fluidic tube couplers

There is often a need for tube connectors on a microdevice for interfacing between the device and the outside world. The main demand on such connectors is that they are leak-tight. Other demands on the couplers depend on the application they are to be used in, but typically include mechanical strength, small footprint, easy and cheap assembly and low dead volume [7,8]. Because efficient standardised connectors are not available, a new and simple melt-on method for conveniently fixing external tubes to the chip was developed and is shown in Figure 8. Mechanical strength and ease of assembly are the main requirements in their specific application as test structure connectors. The connectors have been successfully used for interfacing to the microstructures presented in papers 1-5 and 9.
3.5.3. Interfacing highly parallel devices

Microfabrication features the possibility of process and functionality parallelisation. However, a large number of process locations require a large number of fluids to be interfaced to a small device. Specific requirements on such an interface include absence of cross-contamination, ease of fabrication, cost effectiveness and low chip area consumption. One such parallelised structure, a filter chamber array that contains a novel fluidic interface fulfilling these requirements, is presented in paper 5 and discussed in more detail in chapter 5. Generally, interfacing may be considered the biggest challenge in design of parallel microfluidic analysis devices.
4. Design of reliable diffuser micropumps

This chapter is an overview of the work in this thesis performed in the field of diffuser micropumps. It focuses on the influence of the pump design on the performance and tells the story of the research aimed at improving the reliability of such micropumps.

4.1. Introduction

Numerous micropumps have been developed, featuring many pump principles and manufactured in different materials [9,10]. The research focus in this work lays on reciprocating valve-less diffuser pumps [11]. Such pumps consist of a pump chamber with an actuator-driven diaphragm and flow-directing diffuser elements at the in- and outlet of the chamber. The pump principle is illustrated in Figure 9. The dependency of a diffuser’s flow resistance on the flow direction results in a net flow through the device during one pump cycle.

![Figure 9. An illustration of the pump principle of a reciprocating diffuser pump.](image)

The planar pump design makes it very easy to fabricate as a silicon-glass stack or as a plastic replica. It contains no moving parts, eliminating wear and fatigue, and the lack of small cross-sectional areas reduces the risk for clogging. The performance in terms of delivered flow and pressure makes the pump attractive for a wide range of applications.

An important requirement for liquid pumping and a challenge in the field of micropumps is reliability. Reliability involves pumping during a long period without a device performance degradation caused by small particles or gas bubbles in the pumped liquid. In general, diffuser pumps do not suffer from particle sensitivity since they have no moving parts or small flow channel geometry. However, they are very sensitive to gas bubbles for a number of reasons. The compressibility of gas bubbles in the pump chamber consumes the volume change that should drive the liquid during operation and thus absorbs the actuator functionality. This is illustrated in Figure 10. Moreover, gas bubbles tend to stick at locations close to the diffuser in- and outlets. There, they change the effective diffuser geometry, the effect of which is unpredictable. This is illustrated in Figure 11. Quoting D.S. Miller on the sensitivity of diffuser behaviour on geometric details [12]: "No matter how much attention is paid to the geometrical arrangement [of a diffuser] ... the flow distribution in a system is unique". Earlier reported liquid
diffuser pumps [13] require an extensive priming procedure and de-gassing of the liquid, making them unsuited for many practical applications. The main objective of the research in this work was to solve these reliability issues.

Figure 10. Illustration of the actuation buffering functionality of gas bubbles.

Figure 11. Drawing of a diffuser neck with a gas bubble (white). The arrows, indicating flow lines, show how the presence of a bubble can change the flow pattern in the diffuser.

4.2. Improving the pump design

Small device dimensions make it typically complicated or even impossible to perform measurements of the physical conditions inside microdevices. Therefore, microsystem designers often use model predictions, simulation results or even plain intuition. To perform a theoretical analysis of the bubble creation process in the diffuser pump chamber, e.g., there is a lack of knowledge on the pressure and flow distribution inside the pump device, especially because of the dynamic pneumatic conditions. However, one specific property was thought to influence the pump reliability, namely the compression ratio of the pump chamber.

4.2.1. The compression ratio

It was assumed that by increasing the pump’s compression ratio $\Delta V/V$ (where $V$ is the chamber dead volume and $\Delta V$ the diaphragm volume displacement), a higher relative amount of liquid renewal would remove vapour traces from inside the pump chamber. The gas bubble formation process mainly results from the (time demanding) coagulation of small vapour traces to larger bubbles, which then stick to the inner pump surface. Decreasing the average time vapour traces are present in the pump chamber should reduce this coagulation process. Moreover, a high compression ratio should overcome the buffering action that gas pockets create [14].
An increase of the pump chamber compression ratio $\Delta V/V$ can be obtained by decreasing the chamber dead volume $V$ and increasing the diaphragm volume displacement $\Delta V$. Keeping the pump chamber shallow, which can be obtained by using proper fabrication parameters, creates the small dead volume. To obtain suitable (deep) diffuser dimensions, however, the diffusers and the diffuser in- and outlet were machined deeper than the remainder of the (shallow) pump chamber.

### 4.2.2. Towards improved actuation

A qualitative study of the diaphragm-piezodisc bimorph pump actuator was performed to optimise the diaphragm amplitude and thus the volume displacement per pump stroke $\Delta V$. A full model of the piezoelectric pump actuator requires a coupled field analysis, however. The driving voltage is coupled to the diaphragm’s mechanical deformation via the piezoelectric element and the mechanical movement of the diaphragm in its turn is coupled to the pneumatic conditions inside the pump chamber. Because of the model complexity, we opted for studying a simplified model of the diaphragm-piezodisc bimorph [15]. The model is illustrated in Figure 12. It consists of a stack of a circular diaphragm and a piezodisc with equal diameter that is simply supported at its boundary.

![Figure 12. Simplified model of the pump’s piezoelectric bimorph actuator.](image)

The pneumatic conditions in the pump chamber are not taken into account. Also, dynamic effects are omitted (the latter probably being largely determined by the pump chamber’s pneumatic conditions). The analytical formula for the static bimorph deformation can be written as [15]

$$w(r) = \frac{R^2 - r^2}{t_p^2 \left[ 3(1 - \eta^2) + 4(1 + \eta)(1 + \eta^2) \right]} \left[ 3d_{33}(1 + \eta)\alpha U \right]$$

and the volume change as

$$\Delta V = 2\pi \int_0^{t_p} w(r) r dr = \frac{3\pi d_{33}(1 + \eta)\alpha U RF}{2t_p \left[ 3(1 - \eta^2) + 4(1 + \eta)(1 + \eta^2) \right]} \cdot$$

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R$</td>
<td>the overall radius</td>
</tr>
<tr>
<td>$t_p$</td>
<td>the piezodisc thickness</td>
</tr>
<tr>
<td>$U$</td>
<td>the voltage over the piezodisc</td>
</tr>
<tr>
<td>$\nu$</td>
<td>Poisson’s ratio</td>
</tr>
<tr>
<td>$d_{33}$</td>
<td>the piezoelectric charge constant</td>
</tr>
<tr>
<td>$s_{11}^E$</td>
<td>the elastic compliance</td>
</tr>
<tr>
<td>$E$</td>
<td>the Young’s modulus of the diaphragm material</td>
</tr>
</tbody>
</table>

Table 3. Parameters used in the pump actuator model.
An explanatory list of symbols is given in Table 3. Despite the simplifications, the formulae above relay information about the influence of the bimorph layer dimensions and preferred diaphragm material. The volume change formula indicates the strong dependency of the pump stroke on the diaphragm radius ($\Delta V \sim R^4$). To allow comparison with earlier investigated structures [13], however, the novel pump design had an unchanged diaphragm diameter of 6mm.

Contour plots of the bimorph centre deflection as a function of the piezodisc thickness and the diaphragm thickness are shown in Figure 13.

![Contour plots of the bimorph centre deflection as a function of the piezodisc thickness and the diaphragm thickness.](image)

**a. Silicon as the diaphragm material**  
**b. Glass as the diaphragm material.**

*Figure 13. Contour plots of the bimorph centre deflection as a function of the piezodisc thickness and the diaphragm thickness. Silicon and glass are used as diaphragm materials. The straight line indicates the optimal diaphragm thickness for every given choice of piezodisc thickness.*

For every specific piezodisc thickness (horizontal line on a plot), an optimal diaphragm thickness in terms of bimorph deflection can be determined. The straight line on the plots connects these maximum deflection points. This interpretation of the plot is important when a piezodisc of fixed thickness must be chosen (in our case we had to choose from a product catalogue). A first conclusion can be drawn concerning the preferable diaphragm material. For a specific piezodisc thickness, silicon diaphragms with optimal thickness give a larger bimorph deflection than glass diaphragms. Secondly, the plots show that thin diaphragm and piezodisc values are preferred. When a piezodisc of a fixed thickness needs to be chosen, however, the silicon diaphragm thickness should be 32% of the piezodisc thickness. The latter can be seen as the slope of the optimum line in Figure 13a. The thinnest piezodiscs found were 100µm thick. Therefore, instead of using a 500µm thick glass diaphragm in the bimorph [13], a 32µm thick silicon diaphragm is used.

### 4.3. Fabrication

The new design, featuring a shallow pump chamber and an optimised diaphragm thickness, is illustrated in Figure 14. For the construction of this pump, two new fabrication sequences were
Designing Microfluidic Control Components

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developed. The first is a method that allows to consecutively deep reactive ion etch (DRIE) three different etch depths in a silicon substrate without the need for repatterning the surface between the etch steps. This is obtained by applying and patterning the three different masking materials (silicon dioxide, aluminium and photoresist) prior to the three etching steps. Between every etch step, one mask material is selectively removed. In this manner, no complicated patterning is needed on non-planar machined surfaces. The second new fabrication method includes the application of fluid connectors to the chip substrate, which is described in subsection 3.5.2.

4.4. A new behaviour

The novel design resulted in more than reliability improvement alone. The position of the piezoelectric disc on the silicon side rather than on the glass side [13] enables observation of the liquid during pumping. Visualisation of the flow patterns was done with the help of small particles in the liquid. In oscillating flow and at locations with high flow velocities, e.g. in and around the diffusers, the visualisation particles are observed through a microscope as lines. These lines have a distinct beginning and end. Their length is a direct indication of the (dynamic) flow velocity; their movement indicates the (static) net flow at a certain position in the pump and shows that the flow regime is laminar.

4.4.1. To be or not to be (a diffuser)

A first observation was that the optimised bimorph actuator makes it possible to pump at frequencies different from the resonance frequency. A second observation was the bidirectional pumping, the pump direction being controlled mainly by the pumping frequency. Bidirectionality had been presented earlier for valve pumps [16,17], where the phase shift between two or more mechanically moving parts determines the flow direction. For diffuser pumps, however, such explanation is not valid. Somehow, the flow properties of the diffuser elements dramatically change with the pump frequency and amplitude, which raises the question whether the diffusers really function as diffusers.

At the time of publication of papers 1 and 2, no answers could be formulated to this question. With hindsight, and after many more hours of staring in a microscope, some more light can be shed on the mystery. Generally, the diffuser micropump principle builds on the fact that the diffuser elements have
a lower flow resistance in the forward than in the reverse direction. (The term forward refers to the direction of increasing diffuser cross-sectional area, while reverse refers to the opposite direction.) The efficiency of the elements can be expressed as \( \eta = \frac{\xi_{\text{reverse}}}{\xi_{\text{forward}}} \), where \( \xi_{\text{reverse}} \) and \( \xi_{\text{forward}} \) are the pressure loss coefficients in the forward and reverse directions, respectively [11]. For diffusers with values of \( \eta > 1 \), the flow resistance is lower in the forward direction than in the reverse, while elements with values \( \eta < 1 \) have a flow resistance that is lower in the reverse direction. Diffusers designed for a net forward pump action, i.e. with values of \( \eta > 1 \), should show optimal pressure recovery (and thus minimal losses) in the forward direction. In macroscale diffuser devices, such flow occurs in the transitory stall region [18]. The forward loss coefficient \( \xi_{\text{forward}} \) of the element is strongly dependent on the flow conditions in the element, however. If flow conditions occur for which the pressure recovery in the forward diffuser direction is poor, \( \xi_{\text{forward}} \) might increase considerably, and the overall diffuser efficiency value \( \eta \) might drop below unity. Such element will cause a net reverse flow if it is used as a flow directing pump element in a reciprocating pump [19]. During liquid pumping, it was observed that under certain oscillating flow conditions different regions of the same diffuser element simultaneously contain a net flow in opposite directions. This indicates that the diffuser flow losses are indeed very different from losses at optimal flow conditions.

Flow visualisation during gas pumping was not performed because there is no easy manner available to do this. It was noticed, however, that the peak pumping performance for both forward and reverse gas pumping occurs very close to the resonance frequency. This indicates that the pump amplitude, and thus the flow speed in the diffuser, strongly determines the diffuser behaviour. Perhaps at resonance, jet flow occurs in the diffuser element in which the flow separates completely from the diffuser walls [12], causing the resulting loss coefficient ratio \( \eta \) to drop below unity.

4.4.2. The dance of the bubbles

A next observation is that vortex-like flow patterns occur in the pump chamber. Their shape, rotation direction and speed are dependent on the actuation frequency and amplitude. Another observation is the formation of gas bubbles due to cavitation in the centre of the pump chamber, which can be observed at almost every pump frequency. It is unsure whether the dynamic pressure drop in the diffuser gives rise to cavitation as well.

Figure 15. Schematic drawing of some of the observed flow patterns in the pump chamber, from left to right at increasing actuation frequency. The black arrows symbolise the diffusers. The grey arrows show the observed flow vortices. The white bubbles indicate typical low-velocity locations in the pump chamber where bubble stiction occurs.
The flow patterns play an important role in the pump’s bubble tolerance. First, the drag of the high velocities occurring in the flow vortices ensures that large bubbles are torn apart into smaller bubbles that do not stick so easy to the inner pump surface. Moreover, only certain locations inside the pump chamber have flow velocities low enough to allow the bubbles to stick to the inner surface. A drawing of the flow patterns and the observed bubble stiction regions is shown in Figure 15. The bubble trap near the diffusers is at least partly caused by the two-level pump chamber geometry. The semicircular deeper pump chamber regions, located where the diffusers are connected to the pump chamber, form a preferred location for bubble stiction. The effect of this is twofold; bubbles are more likely to be transported out of the pump chamber because of their location close to the diffusers, but on the other hand, bubbles can actively change the diffuser geometry, as illustrated in Figure 11. A general observation is that the new design enables bubble-tolerant pumping of liquids.

An overall overview of the new pump design and its influence on pump behaviour and pump functionality is given in Figure 16.

Figure 16. Schematic overview of the new pump design and its influence on pump behaviour and pump functionality.

### 4.5. A look ahead

As often, opening up one bottleneck shows you the next one. The inherently stiff piezodisc is fixed to the silicon diaphragm with (soft) glue, which is not optimal from a mechanical strength point-of-view. When relatively large amounts of gas are present inside the pump chamber or when pumping pure
gas, the large bimorph deflections heat up the structure, which results in glue failure. The limited lifetime of the structures poses a severe problem for long-term pump actuation.

Novel pump research should thus focus on reliable actuation. Enlarging the pump diaphragm diameter or changing the actuation principle could be a first step in that direction. The need for a large pump stroke, combined with the general bubble problems caused by microscale surface tension phenomena, indicate that reciprocating diffuser micropumps for liquids might be interesting components on a milliscale rather than on a microscale.
5. On-chip fluid control for biotechnical and chemical applications

“One pressure to rule them all,
Hydrophobic patches to find them,
Tiny pillars to catch the beads,
And in the microchip bind them.”

(Freely adapted from [20].)

5.1. Introduction

One area for microfluidic liquid handling lays in medical, biotechnical and pharmacological applications, where on-chip nanoliquid sample control forms the base for a range of applications with a huge market potential. Downscaling biotechnical and chemical processes has a large number of advantages. Depending on the application, it may lead to less reagent consumption, lower equipment manufacturing cost, increased performance, faster analysis, higher sample throughput, easier integration and automation, and disposability. A detailed overview and references on microfluidic devices for biotechnology and organic chemical applications can be found in other work [21,22].

5.2. Surface based systems and closed channel systems

A number of surface-based microarray systems have been shown where drops of samples and reagents are deposited onto a substrate. The simplest design involves deposition directly onto the substrate. Other designs incorporate microvials and/or hydrophobic surface treatment and/or immobilised beads. The main advantage with using open structures is the ease with which samples and reagents can be added or withdrawn. Other advantages are the large number of spots, the ease of fabrication and the lack of fluidic interconnections. Disadvantages include fast liquid evaporation, sample and reagent concentration control, contamination from the outer environment (dust, including biological material) and sample (cross-)contamination.

In channel based microfluidic systems, pneumatic pressure can be used for liquid control and typical surface related effects, described in chapter 3, are more prominent than in open systems. The greater control of material location makes channel based systems therefore generally more versatile than surface based systems. Moreover, channel based systems provide a natural solution to the typical limits of the surface based systems including evaporation and (cross-)contamination. Disadvantages
include the more complicated interfacing and liquid control. Table 4 gives an overview of some characteristic properties for both types of system.

<table>
<thead>
<tr>
<th></th>
<th>Surface based systems</th>
<th>Channel based systems</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liquid handling</strong></td>
<td>Some experimental systems [2,23], not well elaborated</td>
<td>Many possibilities</td>
</tr>
<tr>
<td><strong>Functionality</strong></td>
<td>Rather limited but highly parallel</td>
<td>More versatile</td>
</tr>
<tr>
<td><strong>Contamination</strong></td>
<td>Sensitive</td>
<td>Requires appropriate interface design</td>
</tr>
<tr>
<td><strong>Evaporation</strong></td>
<td>Problematic, careful control required</td>
<td>Unproblematic</td>
</tr>
<tr>
<td><strong>Interfacing</strong></td>
<td>Macro- and microdispensing techniques</td>
<td>More complicated, area consuming</td>
</tr>
<tr>
<td><strong>Fabrication</strong></td>
<td>Relatively easy</td>
<td>More complicated</td>
</tr>
</tbody>
</table>

*Table 4. Comparison between the characteristic system properties of surface based and channel based systems.*

Controlling chemical and biotechnical processes in a channel based microfluidic system includes:

1. entering the samples and reagent liquids on the chip
2. confining the samples to specific on-chip locations
3. time controlling processes
4. collecting the process results and
5. removing waste products.

Steps 1, 3 and 5 involve interfacing between the *Macroworld* and the microdevice. Steps 2, 3 and 5 require control of well-defined liquid volumes both in space and in time. Step 4 requires a suitable detection technique for the results of the chemical processes.

The following sections describe the design and fabrication of a flow-through microdevice for chemical and biotechnical processes, presented in papers 3, 4, 5 and 9. The fluidic functionality of the device is focussed on in this chapter and the choice of specific design elements is clarified. For those that prefer easy reading, I would like to refer to the Appendix, which contains a popular-scientific article overviewing this part of the research.

### 5.3. Confining on-chip chemical processes

To control a large number of chemical processes on a single microdevice, one needs the ability to define the exact process location. Traditionally, non-flow-through recipients are used for this purpose. However, a problem often encountered in chemistry is that reaction products and waste products must be separated after a chemical process. Using a solid phase, to which the chemical samples of interest bind and to which liquid reactants can be added and from which reaction products can be removed easily, enables this separation.

To avoid cross-contamination and reagent evaporation, the fluidic system must be a closed channel system. The microdevice is therefore designed as a flow-through cell in which the sample is confined.
Designing Microfluidic Control Components

onto specific on-chip locations. Binding the sample directly to the chip surface, e.g. using array-spotting techniques, could be used for this purpose. This step must be performed during the device fabrication, however, before the device is closed. This complicates the microfabrication and narrows the use of each device to one specific application. To overcome this limitation, the use of beads as the mobile solid phase was chosen. Beads are microspheres on which samples can be bound and which are routinely used in a large number of biochemical applications. A SEM picture of beads is shown in Figure 17.

![SEM picture of beads with a 5.5µm diameter.](image)

**Figure 17. A SEM picture of beads with a 5.5µm diameter.**

Confining the beads to a specific on-chip location confines the sample. Some have shown bead confinement using magnetic beads and a magnetic field [24,25]. To avoid integration of magnetic components on or to the chip, a different approach was taken in this work. A filter chamber was designed, consisting of a grid of tiny pillars, which forms a mechanical barrier for the beads in the flow-through cell while allowing liquid to pass freely through the cell. The shape of the filter device defines the reaction chamber volume, minimises the risk for clogging and adapts the process location to the shape of the detection sensors, e.g. the rectangular camera pixel shape in pyrosequencing applications. Figure 18 shows some SEM pictures of a filter chamber.

![SEM pictures of the fluidic wafer part of a filter chamber device before bonding with a coverlid.](image)

**Figure 18. SEM pictures of the fluidic wafer part of a filter chamber device before bonding with a coverlid.**

The device is fabricated as a two-wafer stack. The bottom wafer defines the fluidic system, including fluidic interfacing, channels, surface modifications and filter pillars. Bonding it to a (glass) cover wafer closes the system. In pyrosequencing applications, the chemical reactions produce photons, which pass through the glass cover where a CCD camera detects them. For other applications, the system can easily be adapted to other detection methods, e.g. fluorescence, or measurement of a change in physical or chemical properties of the liquid. For such purpose, a wafer containing a detection system can replace the glass cover wafer. This is illustrated in Figure 19. Low temperature wafer bonding,
using BCB e.g. [6], is still required to ensure that the hydrophobic control valves are not destroyed. Therefore, the detection wafer bonding does not complicate today’s wafer bonding process.

**Figure 19. Illustration of a) today’s design and b) how it can be adapted to other detection principles.**

### 5.4. The interface

For high-speed screening purposes, process parallelisation is desirable. Therefore, a single device containing a multitude of identical filter chambers was fabricated as described in paper 5. Because of the interfacing problems (described in subsection 3.5.3), tube coupling was not used for sample and reagent delivery to the chip. Instead, contactless liquid delivery was chosen. A number of microdroplet generators are available on the market, designed for microdispensing purposes [26,27]. One advantage of contactless liquid delivery is that the chip is pneumatically de-coupled from the delivery system, which makes both systems independent of one another, avoiding all practical problems related to system integration. Note that the liquid delivery system is beyond the scope of the work presented.

Whereas the different samples to be analysed in parallel typically differ from one another, the reagents they react with are the same for all reaction locations. The chip inlet interface is therefore designed to allow both individual access to every reaction location (for sample loading) and access to all reaction locations simultaneously (for reagent loading), the latter being easier and less time-consuming to accomplish. This is illustrated in Figure 20. Note that this interface design also uses surface tension effects to allow temporary liquid storage on top of the chip substrate. This type of storage volume is larger than that in designs where the liquid is stored inside the device because of the typical planar design of microstructures.

The control of the on-chip hydrophobic valves is obtained through the coupling of an external pneumatic signal. This is provided via the single common waste outlet of the system. Liquid control and waste outlet are thus provided through one single coupling element.
Separate samples are added to different reaction locations.

Surface tension increases on-chip liquid storage.

On-chip channels

One reagent is added to all reaction locations simultaneously.

A hydrophobic chip surface confines the reagent volume.

- **a. Small liquid quantities only serve one inlet.**
- **b. Large liquid quantities serve all inlets simultaneously.**

**Figure 20.** The device interface allows liquid delivery to both specific reaction locations (a) and to all reaction locations simultaneously (b).

### 5.5. Controlling on-chip fluid movements

Since the fluid control of every fluidic cell is identical, the system is designed to control all samples simultaneously. A schematic overview on how this is achieved is shown in Figure 21.

A reagent is introduced to all reaction cells on the chip. A “weak” hydrophobic patch, i.e. having a small threshold pressure, prevents the reagent from entering the reaction chamber.

A small pressure signal at the common outlet allows the reagent to enter the reaction chamber and start the reaction. The “strong” hydrophobic patch prevents the light emitting reaction products from exiting the reaction chamber.

In order to remove waste products and prepare the reaction chamber for the next cycle, a strong pressure signal at the common outlet evacuates all liquid.

**Figure 21.** Illustration of how one pressure signal controls the movement of all on-chip liquid samples. One reaction cycle is illustrated.

In order to control a large number of samples simultaneously, the design must allow a “digital” fluid control, i.e. featuring insensitivity to pneumatic or fluidic “noise”. Hydrophobic patches with specific
breakdown threshold pressures offer this. Weak and strong hydrophobic patches with respective low and high threshold pressures $P_{th,\text{low}}$ and $P_{th,\text{high}}$ divide the pressure control range in three regions. All pressures below $P_{th,\text{low}}$ do not influence the on-chip liquid position. All pressures between $P_{th,\text{low}}$ and $P_{th,\text{high}}$ allow liquids to enter the reaction chamber via the inlet but not to exit it. All pressure above $P_{th,\text{high}}$ will transport all fluids to the common waste. Note also that the strong hydrophobic patch at the chamber outlet prevents capillary backflow of fluids from waste to reaction chamber. Using one single tube connector for the complete chip as both common waste outlet and pressure signal interface minimises the requirements on the microsystem interface.

To create the hydrophobic patches in the liquid duct, a novel fabrication method was developed. It is based on a lithographically defined plasma deposited C$_4$F$_8$ layer with a contact angle $\theta_c=108^\circ$, as described in paper 4. The channel geometry, defined during the fabrication, determines the exact threshold pressure of the patch. A fabrication method involving a minimum number of lithography steps thus allows the fabrication of a complete micro analysis system.

So far, the interface and control method have been successfully tested using an array of three filter chambers with hydrophobic patches at each filter chamber inlet. At the time of writing, research focuses on the reliability and fabrication of filter chamber systems with both weak and strong hydrophobic patches.
6. Microvalve control of gas flow

During a wander on the Internet I bumped into the picture shown in Figure 22. It illustrates that certain people give a rather odd meaning to the word “microvalve”. This chapter overviews the work presented in this thesis in the micro gas flow control field, where device features are given in micrometres rather than millimetres.

Figure 22. Found on the Internet: “The Schmidt MicroValve may be small in physical size, but its efficient, low profile design performs in a big way.”

6.1. Size, cost and performance

Commercially available microvalves are expensive and focus therefore on niche markets where typically component size reduction outweighs cost increase, or where semiconductor valve materials can withstand harsh gas environments. The company Redwood Microsystems™, for example, lists performance benefits of its microvalves including proportional control, integration capabilities, microflow capabilities (less than 1sccm) and a wide dynamic range (1500:1 flow ratio), while it suggests as possible application areas analytical instrumentation, medical instrumentation, process control equipment, I/P converters, pressure and flow regulators, pneumatic controls, HVAC controls, test and calibration equipment and gas mixers.

The author believes that the microvalve industry can be successful outside the specific niche markets it currently focuses on if the fabrication cost can be reduced to a level below that of standard (solenoid) valves while delivering the same pneumatic performance. The work in the gas control field presented in this thesis therefore focuses on component cost reduction through miniaturisation.

The general challenge in gas valve design is dual. First, pneumatic applications often require the control of high forces. However, for every type of actuator, there exists a limiting maximum work per actuator volume that can be delivered. Either the actuator stroke-length or the force delivered by the actuator is therefore limited. Secondly, the small cross-sectional areas of fluid ducts in microdevices limit the flow. To reduce the component size of microvalves without reducing the pneumatic performance, these two major problems must be addressed.

The majority of earlier presented microvalves contain an out-of-plane moving boss element that regulates an out-of-plane gas flow [28-31,33-35]. The overall performance in terms of pneumatic
control of such out-of-plane valve actuators can be qualitatively described with the pressure ($P$) stroke ($z$) product of the actuator, $P \cdot z$, because the actuator stroke is typically related to the flow the device can handle. Pneumatic performance improvement of microvalves can thus be obtained either by increasing the delivered actuator performance, or by decreasing the required actuator performance. The first strategy is followed in the design of a novel electrostatic valve actuator, presented in paper 7 and discussed in the next section. The second approach is used in the design optimisation of microvalve nozzles as presented in paper 8 and discussed in the last section of this chapter.

### 6.2. A novel electrostatic pressure-balanced microvalve actuator

Electrostatic actuators are used in many microsystem designs, including microvalves. The quadratic force increase for decreasing electrode distance makes electrostatic forces typically suitable in microsystem actuators. The rapid force decrease for increasing electrode distance, however, puts a severe limit to the actuator’s stroke-length. Pressure balancing has been proposed as a solution for this problem in valve actuators [31]. It reduces the actuation force needed with a proper geometrical design. Pressure balancing solves the limited stroke-length problem only partly, however, and

![Diagram of the novel valve actuator and of the actuator diaphragm functionality and the requirements this poses on the design.](image)

**a. In the closed valve state, a small downward electrode deflection at the diaphragm edge allows easier electrostatic actuation.**

**b. In the open valve state, a large diaphragm centre deflection ensures a larger valve opening.**

*Figure 23. Schematic of the novel valve actuator and of the actuator diaphragm functionality and the requirements this poses on the design.*
relatively high actuation voltages are still needed. A novel actuator concept was therefore developed and is illustrated in Figure 23. Its working principle is illustrated in Figure 24.

- **a. No actuation voltage:** The net pressure forces on the boss are downward, keeping the valve closed. The dashed arrows indicate the pressure force.

- **b. Actuation voltage applied:** The diaphragm zips along the fixed electrode. The solid arrows indicate the electrostatic force.

- **c. The valve opens, and the compliance of the diaphragm allows a large gas flow gap.**

*Figure 24. Schematic cross-sectional view of the functioning of the valve actuator concept.*

The valve’s (high) inlet pressure works on both the boss and the actuation diaphragm, while the valve’s outlet is at a lower pressure. Proper area dimensioning of the diaphragm ensures that the closing force that the diaphragm exerts on the boss is higher than the direct pneumatic opening force from the inlet pressure. In the non-actuated state, the valve is therefore closed. Applying an electrostatic actuation voltage between the diaphragm and the fixed electrode results in an electrostatic pressure on the diaphragm. This reduces the force that the diaphragm exerts on the boss. Once the closing force from the diaphragm on the boss is smaller than the opening pressure force from the inlet, the valve opens. While the valve switches to the open state, the edge of the diaphragm zips along the fixed electrode. Due to the compliant nature of the diaphragm, the boss has a large stroke-length, allowing a high gas flow through the slit between seat and boss. Removing the electrostatic forces returns the pressure distribution on the diaphragm to its original state, and the valve closes again.

The diaphragm of the electrostatic actuator thus has three functions, as illustrated in Figure 23, and each function influences the valve design. The first function of the diaphragm is to transfer the balance pressure to the boss. This is ensured if \(4 \cdot A_{\text{boss}} \leq A_{\text{diaphragm,closed}}\), where \(A_{\text{diaphragm,closed}}\) is the free diaphragm area for a closed valve and \(A_{\text{boss}}\) the pressurised boss area [32]. The second function of the diaphragm is that it constitutes the flexible guidance of the boss. Once the net force on the boss opens the valve, the compliance of the diaphragm is the only limit to the boss stroke-length. A large (flexible) free diaphragm area for the open valve state seems therefore desirable. For a certain diaphragm size, however, the balance pressure on the diaphragm counteracts the boss movement. It is shown in paper 7 that the free diaphragm area for optimal stroke-length can be found in the relation \(A_{\text{diaphragm,close}} = 2 \cdot A_{\text{diaphragm,open}}\), where \(A_{\text{diaphragm,open}}\) is the free diaphragm area for the open valve.
The third function of the diaphragm is that it constitutes a flexible actuation electrode. When the valve is closed, a maximum electrostatic actuation force is desired and the electrode gap should thus remain small. The balance pressure deflects the electrodes away from one another, however, and minimum diaphragm stiffness is therefore necessary to limit the electrode deflection. Limited diaphragm deflection means also limited actuator stroke, however. The optimal diaphragm thickness can be defined as the minimum thickness that still allows electrostatic pull-in. A practical manner to define this thickness is to simulate the actuator behaviour for decreasing diaphragm thickness until the electrostatic forces cannot pull in the valve diaphragm any longer. Additionally, one should also consider the mechanical diaphragm strength and fabrication and operation tolerance before deciding on the final fabrication parameters. The choice of the actuator design parameters is outlined in Figure 25.

Another aspect that should be taken into account is the mechanical stability of the system. A valve configuration as shown in Figure 23 and Figure 24 where the boss is supported by a single flexible diaphragm might be mechanically unstable. A design where the boss is supported by three actuator elements is shown in Figure 26. Such a design is expected to be more robust than a single-diaphragm design.
6.3. Smaller features with larger flow

This section describes how optimising a microvalve nozzle can improve the valve’s flow capacity and diminish the required actuator performance. It sounds contradictory, but the study presented in paper 8 concludes that feature size reduction can lead to improved flow handling capability in microvalve design.

A typical valve contains a boss, suspended over an orifice in a substrate. The movement of the boss controls the flow through the device. Such a configuration is shown in Figure 27.

The feature size of earlier reported valve structures with a similar design [28-31,33-35] and an analysis of the flow resistance along the flow path through those structures shows that, especially for valves with a low stroke-length, the main flow restricting area is most often located at the valve seat instead of in the valve orifice and that the actuator size, rather than the flow-path dimensions, determines the overall device size.

Therefore, a novel design strategy was developed that allows an increased mass flow per unit of orifice area, as well as a decreased actuator stroke-length. The strategy involves enlarging the valve seat area until the flow becomes orifice-controlled rather than seat-controlled. Once the flow is orifice-controlled, a maximum mass flow per unit of orifice area is obtained. In an orifice-controlled flow design, the boss stroke-length does not influence the valve flow and can thus decrease. In the novel design, the single boss–orifice configuration, illustrated in Figure 27, is replaced by k identical boss-orifice configurations that are downscaled by a (linear) factor \( \sqrt{k} \). Figure 28 shows an illustration of such a design.
The overall seat area of the novel design is increased with a factor $\sqrt{k}$, while the overall orifice area remains constant. The novel method for seat-area increase is illustrated in Figure 29.

Figure 28. Cross-sectional view of a multiple-orifice valve nozzle.

Figure 29. Schematic overview on how the miniaturisation and multiplication procedure keeps the total orifice area constant while increasing the total seat length.
To prevent frictional flow losses in the downscaled substrate orifices, the substrate can be thinned down. Limits for the boss and orifice multiplication and miniaturisation process are discussed in paper 8 and include clogging sensitivity, manufacturability, leak-tightness and the mechanical strength of the substrate. The complete design guidelines are outlined in Figure 30.

![Diagram](image)

*Figure 30. Schematic overview of the design guidelines for microvalve flow optimisation following the above optimisation strategy.*

Paper 8 also presents a detailed study, using Finite Element Analysis (FEA) and analytical flow predictions, of the influence of the geometrical design parameters on the flow capacity of the novel orifice design.
Figure 31 shows that orifice-controlled flow can also be implemented in a more complex 3-way valve. The design features pressure balancing for both the high-pressure and low-pressure port. Note that in this specific design, the pressure balancing functionality and the actuation functionality are physically separated.

Figure 31. Schematic of a 3-way valve featuring orifice-controlled flow and pressure balancing. The valve connects a working volume (2) with a high-pressure source (1) in the off-state. In the on-state, the working volume (2) is connected to a low-pressure port (3) for gas evacuation.
7. Conclusion

A number of new microfluidic components were designed and investigated for a range of different application areas. A part of them were fabricated and successfully tested.

Specifically, in the micropumps research field, a new micro diffuser pump was designed, fabricated and tested. Gas bubble tolerant pumping was shown for the first time for this type of pump. The pump is also the first bidirectional reciprocating micropump without mechanical valves. Furthermore the device can handle gas as well as a wide variety of liquids that are of interest in biotechnical applications. Pumping of beads and living cells was successfully performed. The existence of complex flow patterns inside the micropump chamber was shown.

In the biotechnics research field, a novel device for on-chip (bio-)chemical processes was developed, fabricated and successfully tested. Its design includes a mechanical filter for bead trapping and a novel fluidic interface. A new liquid control method is presented with minimal fluidic interfacing between the Macroworld and the microdevice. These features allow simultaneous control, both in location and in time, of a large number of parallel on-chip chemical processes.

In the microvalves research field, two new concepts were developed and proven. A novel electrostatic gas valve actuator was designed, promising a significant performance improvement in terms of pneumatic control. It was also shown that a proper microvalve nozzle design allows a drastic increase of the flow capacity and a decrease of the required actuator stroke of a microvalve.

Finally, a new concept for a pressure-driven energy converter was introduced and studied.
8. Summary of appended papers

The appended papers 1 to 8 contain the publications of the author in the field of microfluidic design.

Paper 1

In this paper, a new micropump design is introduced and test devices are studied. The new pump features a two-level pump chamber geometry and an optimised piezoelectric bimorph actuator that allows an improved pump reliability. It consists of a silicon-glass stack and is fabricated with a new process involving three sequential DRIE (Deep Reactive Ion Etching) steps. A new and simple technique for fluid interconnection was developed. The device is the first reported reciprocating valveless pump that enables both gas and liquid pumping and the first that is bidirectional. The micropump is also fully self-priming and the complex vortex flow patterns occurring in the pump chamber make the device relatively insensitive to cavitation and gas bubbles in the liquid. Changing the actuation frequency enables bi-directional pumping for both liquid and gas, i.e. both forward and reverse pumping. Design, fabrication and first experimental results are described and discussed.

Paper 2

Paper 2 shows the suitability of valveless micropumps in biochemistry. Fluids encountered in various biochemical methods that are problematic for other micropumps were pumped with good performance. Some of the main advantages of the valveless diffuser pump are the absence of moving parts (excluding the pump diaphragm), the uncomplicated planar design, and high pump performance in terms of pressure head and flow rate. In addition, the micropump is self-priming and quite insensitive to particles and bubbles present in the pumped media. The results show that the valveless micropump successfully pumps fluids in the viscosity range of 0.001-0.9Ns/m². It is not sensitive to the density, ionic strength, or pH of the pumped media. Effective pumping of solutions containing beads of different sizes was also demonstrated. Living cells were pumped without inducing cell damage and no cell adhesion within the pump chamber was observed. No valveless micropump has previously been reported to pump such a wide variety of fluids.

Paper 3

This paper discusses the design, manufacturing and characterisation of a new flow-through micromachined device for chemical processes on beads. The device can collect beads in a reaction chamber with a filter grid that forms a mechanical barrier. It features an uncomplicated planar design and microfabrication process. The sample flow-through volume of liquid or gas is adjustable and unlimited. The device is sealed with Pyrex to allow real time optical detection of the chemical reactions. At a constant pressure of 3kPa at the inlet, the flow rate for water is about 3.5ml/min without beads in the filter chamber for all the designs. The smallest reaction chamber has a volume of 0.5nl and can collect approximately 5000 beads with a diameter of 5.5µm. When the reaction chamber is completely packed with beads, the flow rate decreases with about 40%. The flow-through microfluidic
device is not sensitive to gas bubbles, and clogging of the filter is rare and reversible. The beads are easy to remove from the reaction chamber, making the micromachined flow-through device reusable.

**Paper 4**

The suitability of using octafluorocyclobutane (C₄F₈) patches as hydrophobic valves in microfluidic biochemical applications is shown in this paper. A technique was developed that generates lithographically defined C₄F₈ hydrophobic patches in DRIE silicon channels. Some of the advantages of this process are that no specific cleaning of the substrate is required, C₄F₈ is deposited on the sidewalls and the bottom of the channels, a standard photoresist mask can be used to define the patches, and that it is a fast and convenient dry chemical process performed with a standard ICP (Inductively Coupled Plasma) etcher using the Bosch process. The valve function of the hydrophobic patches was tested for the following liquids: DD water, acetone, propanol, bead solution and a mixture used for pyrosequencing of DNA. Patches of C₄F₈ successfully stopped each solution for at least 20 consecutive times. The C₄F₈ film resists water for at least 5 hours. The hydrophobic valve also resists very high concentrations (25%) of surfactants (Tween 80). C₄F₈ shows a much higher resistance towards water and surface-active solutions than previous hydrophobic patches. However, 50% Tween 80 was not stopped at all by the hydrophobic patch. An applied pressure of 760Pa at the inlet was needed for water to over-run the hydrophobic patch in a 50µm sized square silicon channel closed with BCB adhesive bonding.

**Paper 5**

This paper is partly an overview of the work presented in papers 3 and 4 and partly introduces the new concept and design of a filter chamber array, its interface and its control scheme. Also, measurement results of pyrosequencing reactions in the filter chamber are presented in this paper.

The filter-chamber array fabricated enables real-time parallel analysis of three different samples on beads in a volume of 3nL, on a 1cm² chip. It contains three filter-chambers with separate inlets, a passive valve at each inlet channel and a common outlet. The design enables parallel sample handling and time-controlled analysis. The device is microfabricated in silicon and sealed with a Pyrex lid to enable real time analysis. SNP (Single Nucleotide Polymorphism) analysis by using pyrosequencing has successfully been performed in single filter-chamber devices.

**Paper 6**

A new concept for a pressure-driven wireless power supply, containing a micro pneumatic-to-vibration energy converter, is presented in paper 6. The fluidic properties are studied with the help of a simplified model in order to understand the influence of physical parameters on the system’s behaviour. Different phenomena causing the energy conversion are discussed. A test structure was fabricated and measurement results are presented.

**Paper 7**

This paper presents a novel large-stroke electrostatic gas valve actuator for high-pressure applications. The combination of pressure balancing and flexible electrode structures ensures large
actuator strokes at a low actuation voltage. A general procedure for maximising the electrostatic pressure in an electrostatic actuator without suffering from dielectric breakdown is presented. A simulation tool was built to evaluate and optimise the actuator design parameters. The model shows a 5.6 times (theoretical) performance improvement compared to earlier designs. A micromachined test structure was fabricated and evaluated. Measurement results are presented and discussed. Parasitic charging of the actuator was observed and used to further decrease the required actuation voltage.

**Paper 8**

Flow handling capability is a bottleneck for the economical profitability of mass-produced microvalves. The study in paper 8 shows that microvalve nozzles with seat-controlled flow in typical leak-tight microvalve designs perform sub-optimal with respect to the flow capacity, whereas nozzles with orifice-controlled flow enable better pneumatic performance and lower device area consumption. A novel design and optimisation method are presented and discussed. The novel design replaces a single boss-orifice configuration with multiple bosses and orifices. The design reduces the required actuator stroke, which is a key to valve area (and thus cost) reduction. Limitations caused by manufacturability and reliability are discussed. An analytic study and FEA (finite element analysis) of the design’s flow capacity confirm the improved pneumatic properties.

**Paper 9**

In this paper, the principle of allele-specific extension using pyrosequencing chemistry was employed to analyse SNP in the filter-chamber device presented in paper 3. Two SNP sites were selected to evaluate the device. Single-stranded target DNA was obtained by using streptavidin-coated beads. Primers were hybridised to the target DNA and were captured in the filter-chamber. Pyrosequencing reagents including all four nucleotides were applied and an external CCD camera detected the produced light. The bead-trapping device enabled analysis of genetic variations in a reaction chamber volume as small as 12.5nl. The results therefore demonstrate the possibility of performing SNP analysis by this technique in small volumes.
9. Acknowledgements

This work has been carried out at the Microsystem Technology group of the Department of Signals, Sensors and Systems (S3) at the Royal Institute of Technology in Stockholm, Sweden. Financial support was received from the Swedish National Board for Industrial and Technical Development (NUTEK), AB Rexroth Mecman, the Stockholm Foundation of Technology Transfer (TBSS) and the Swedish Agency for Innovation Systems (Vinnova).

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I would like to end this work with the same words as many works have been ended before [36]:

*Bibamus laeti merum, non est mutatio rerum.*

Stockholm, April 2002, Wouter van der Wijngaart
10. Reference list


Appendix

A miniaturized diagnostic tool for biomedical point-of-care applications

The 2002 Innovation Cup award winning contribution of

Helene Andersson and Wouter van der Wijngaart
Appendix - A miniaturized diagnostic tool for biomedical point-of-care applications

Abstract

We present a novel microfluidic chip with a new microfluidic handling method that solves main technological bottlenecks in the development of point-of-care instruments. The main features of the microfluidic chip are: small size (i.e. it is possible to achieve a portable device), less reagent consumption (cheaper analysis), lower equipment manufacturing costs, increased performance, faster analysis, high sample throughput, increased integration and automation, and disposability. The microfluidic chip has been evaluated for the following applications showing promising results: DNA analysis, protein analysis and asymmetric catalysis, which all are of high interest for the point-of-care testing.

While growth in the laboratory testing market is slow, the point-of-care sector is growing at a rate of 27% per year and is projected to continue in this fashion. This demand is attributed to the drive for cost containment in the health care industry. A conservative estimate of the turnover for the invention presented in this report is about US $20 million.

Point of Care Instrumentation

Historically, diagnostic assays are usually done by specially designed clinical laboratories, either on-site or, more typically, out-sourced. One recent trend in diagnostics, however, is an increased popularity of the ‘point-of-care’ (POC) concept. Point-of-care testing is in-situ laboratory testing and analyzing, for example at a patient’s bedside or at a disaster area. Technological advances have facilitated the development of easy-to-use, rapid diagnostic devices that can be used in a POC setting closer to the patient, and that have the ability to pick up disease at an earlier stage. Figure 1 shows an illustration of how a POC system might look. The attraction of POC lays in the immediate result deliverance to the medical practitioner and the patient. The early diagnosis can enable the practitioner to commence sooner treatment and leads to improved patient care. Especially in crisis situations like biological warfare or at a nuclear disaster, such systems will become indispensable in future.

Designers of POC systems face special challenges. The systems should be fast, small and easy to use, but at the same time deliver a reliable state of the art performance. Portability of the device requires that it is small in size, light and battery operated. Other requirements include feature transparency, automatic calibration, and a full automation in its analysis. In addition, the cost of the testing process must be low. In the same way integrated electronic circuits allowed for the miniaturization of the first room-sized computers to today’s
portable PDAs, miniaturization of microfluidics has the potential to shrink a room full of medical instruments into a compact lab-on-a-chip that handles multiple integrated lab processes and is used in a palm-size device (Figure 2). The technology that drives the miniaturization of fluidic systems was originally developed by the microelectronic industry.

However, lab-on-a-chip technology, or micro total analysis systems (µTAS), promises more than just enabling portable POC equipment. It also leads to less reagent consumption, lower equipment manufacturing costs, increased performance, faster analysis, high sample throughput, increased integration and automation, and disposability.

All lab-on-chip applications and solutions proposed require handling and analyzing of liquids in the nanoliter volume range. This extreme downscaling of liquid handling (100 000 times compared to typical microtiter plate volumes) presents numerous technological challenges. Examples illustrating this are the fact that nanoliter volume droplets evaporate in a few seconds if exposed to the open atmosphere, or that surface tension effects make small droplets to stick like glue to whatever surface they get in contact with. The interfacing between the macro and micro world is therefore one of the main problems. Other challenges include the small signal levels that can be expected from these ultra-miniaturized biochemical reactions and the miniaturized geometrical scale of the detection sensors themselves. Solving these challenges opens up a huge scientific and market potential.

**A Novel Microfluidic Chip Concept**

And that is exactly what the MST-group at the Royal Institute of Technology has achieved with this invention. A novel microfluidic chip was fabricated and a new microfluidic handling method was developed, that solves main technological bottlenecks in this research domain [1-3]. The concept’s functionality is as follows. In order to test the biological samples, they are first attached to beads, small microspheres, submerged in a liquid. This method is routinely used in a number of biotechnological and biomedical applications. One benefit of the use of beads is that it prevents the sample from attaching to the internal surface of the microfluidic chip, something that would destroy both the chip device and the analysis of the sample. Another benefit, and key feature in the invention, is that micrometer-scale microspheres are easier to handle than single molecules in a liquid sample. In order to monitor the beads-with-sample during testing, they are confined in a specific volume on the chip. For this purpose, a novel basic microfluidic component was developed: a micromachined filter-chamber, depicted in Figure 3. Note that the silicon device shown in Figure 3 is closed with a glass cover before use, which enables visual observation of the reactions inside the filter chamber.
Appendix - A miniaturized diagnostic tool for biomedical point-of-care applications

Figure 3a. A photo overview of the filter-chamber device.  
Figure 3b. A side view of the filter-chamber.  
Figure 3c. A high magnification of the filter pillars. The pillars are 3x10 µm thick and 50 µm high, and have a spacing of 2µm.

Hundreds of filter-chambers can be batch fabricated onto a single chip. Each filter consists of an array of ultra-thin pillars, defining a square reaction chamber. When the liquid containing the beads is flowing through the filter, the pillars trap all the submerged beads, and in this manner, the sample is confined in a well-defined position on the chip, as illustrated in Figure 4. However, reagents can still flow freely through the filter-chamber, where they can interact with the sample on the bead surface. In the first test series, the reactions in the filter-chamber are visually observed with a CCD camera, as illustrated in Figure 5. The flow-through and timing of the reagents through the filter-chamber is regulated with a pneumatic suction signal at the chip outlet.

Figure 4. A schematic of the filter-chamber device filled with beads.  
Figure 5. Illustration of a measurement with the chip device.

To sum up some of the features of the new device: it allows simultaneous transport and confinement of several different bead samples, parallel handling of reagents, reactions and waste products in a time-controlled manner and this with a minimum of chip interfacing. For a more detailed understand of these features and for a full appreciation, let’s make a comparison with the design of electronic chip.

Digitizing and Clocking contain the Keys to Control

Binary digits, better known as Bits, are the basic information carriers in all modern electronic devices. The reason why digital data communication and processing is preferred over analogue signal processing is that the
information is stored in a different manner in both cases. A bit is physically implemented as a specific voltage level: a voltage higher than a specific threshold represents a binary “1”, while a voltage lower than the threshold represents a “0” (1). In analogue signals on the other hand, information is stored in the exact voltage level of the signal itself. Therefore, in analogue signal processing, every small distortion intrinsically changes the information content of the signal. Digital signals however are completely insensitive for any distortion signal that does not make the bit’s voltage to surpass the threshold value, and digital processing is therefore much more robust. Directly coupled to the use of bits in an electronic circuit is the manner in which the information stream is controlled with a so-called system clock. The clock beats determine when the bits are allowed to move or be processed. At every clock beat, the bits are allowed to pass through one functional block on the processor. Therefore, if two numbers A and B need to be added e.g., the clock ensures that A will not enter the adder block before B has arrived at the adder’s entrance.

The same type of functionality is necessary on a microfluidic chip. The microfluidic “functional blocks” or components consist typically of on-chip liquid storage reservoirs, filter-chambers (reaction chambers) and inlet or outlet channels. A “microfluidic bit” consists of a small on-chip liquid volume (e.g. a reagent, reaction product or waste product). Either the liquid volume is at a certain position in the chip (a binary “1”) or it is not (a binary “0”). Of importance is that one has to have absolute control over the place and movement of these fluid bits. The liquid volumes are confined within the borders of their functional components on the chip within one “clock cycle”, where they can not disturb processes in neighboring components. Moreover, exact control and timing of the chemical reactions that take place is necessary if one wants to interpret the measurement data from the reaction chambers in a correct way. If hundreds of liquid volumes are moving around on one device, a simultaneous time control of all samples is required. E.g., It is necessary to remove waste products before the next sample enters the reaction chamber, and one has to know the exact start and stop time of the reactions.

In the invention, the clocked timing of all the liquid digits is implemented through the combination of capillary suction and hydrophobic materials. Small capillaries, like on-chip channels, tend to suck the liquid into the channels on the chip. However, manipulating their surface geometry or material properties allows creation of hydrophobic regions in the channels that repel the liquid rather than suck it in. Basically, liquid will fill the capillary until it reaches the edge of a hydrophobic region. There it will abruptly stop. The hydrophobic patch forms a well-controlled pressure barrier for the liquid, and therefore acts as a valve. In order to make the liquid to cross the hydrophobic region, one needs to apply a suction to the liquid column that is higher than the pressure barrier of the specific hydrophobic patch. The use of hydrophobic patches with different pressure barriers and the use of a controlled pneumatic suction at the common outlet of the device thus enable a precise control of where the liquid moves and where it is stopped. A comparison between electronic and microfluidic circuitry is illustrated in Figure 6.

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(1) Or vice versa, depending on the technology used.
Schematic of an electronic adder. The green arrows indicate the input and output bits. The component always sums the incoming bit with the last bit stored at its output.

At the clock signal, bit A is allowed to enter the adder and bit B is entered at the input. The adder output is now 0+A=A.

At the next clock signal, bit B is allowed to enter the adder. The adder output is now A+B.

When a pressure signal is applied, reagent 1 enters the reaction chamber and reagent 2 is added at the inlet. Reagent 1 reacts with the sample on the beads, which is observed by the CCD camera (not indicated).

When a next pressure signal is applied, reagent 1 is removed to waste and 2 enters the reaction chamber. Reagent 2 reacts with the sample on the beads, which is observed by the CCD camera (not indicated).

Another analogy between fluidics and electronics lays in the difficulty to interface between the chip and the surrounding world. Interfaces are in both fields very space consuming and expensive in fabrication and must therefore be limited to a minimum. In the invention however, every single reaction chamber needs to be directly addressable through its own inlet on the chip surface to allow the addition of the unique samples under investigation (e.g. one sample per patient) to their respective reaction chamber. However, the reagents that are used are non-specific and need to be added to all reaction chambers simultaneously. Therefore, a novel interface to the chip was developed, as illustrated in Figure 7 and Figure 8. Either every reaction chamber is served
separately with a specific sample or the total of all reaction chambers is served simultaneously with one reagent. The interface for loading the samples consists of small access holes in the chip surface that can be filled with either a micropipet or a micro-droplet generator. A hydrophobic region is surrounding all the separate inlets. Larger droplets of reagents added to the chip surface will be contained within this region and address all inlets simultaneously. Again, hydrophobic strips at the separate channel inlets prevent the common reagent to enter the chip and get in contact with on-chip stored samples, and there is therefore no risk for cross contamination between the different reaction chambers.

There is only one common outlet on the chip for all waste products. This outlet is connected with a tube to a suction pump that delivers the control pressure. In this way the pneumatic clock signal and waste outlet are incorporated in one single interface connection, and all movement of liquids on-chip is controlled through the off-chip suction pressure.

Figure 7. A schematic of one of the devices that were fabricated, containing three filter-chambers. The two key elements, the filter-chamber and the hydrophobic valve, are magnified.

Figure 8. the top part of the figure shows the loading of the unique samples to their respective reaction chambers. A first suction will enter the samples into their respective reaction chamber. The bottom picture shows how a common reagent is loaded and confined by the hydrophobic tape. The on-chip hydrophobic patches prevent the common reagent to enter the chip before a pneumatic control signal is applied at the common outlet.

From a microfabrication point of view, this novel device is uncomplicated and straightforwardly suitable for integration with other devices and/or batch fabrication. The smallest filter-chamber fabricated has a volume of 0.5 nl and can collect approximately 3000 beads with a diameter of 5.50 µm.
Figure 9. A conceptual drawing of a 10 by 10 array of reaction chambers with a grid of channels connecting them to a common outlet.

Note that the above described devices and methods are currently patent pending [4-6].

DNA Analysis

The draft of the human genome, combined with millions of single nucleotide polymorphisms (SNPs, pronounce [ˈsniːps]) that are publicly available enable the pharmaceutical companies to find novel targets and link them to disease. The most common type of human genetic variation is SNPs, a position at which two alternative bases occur at a frequency larger than 1% in the human population. SNPs are relatively common in the genome, are relatively stable and may even be the direct cause of a disease or particular drug-response. There are major efforts to map SNPs throughout the human genome, for example by the commercial/academic run SNP Consortium (http://snp.cshl.org/) and also the privately run efforts of Celera (http://www.celera.com/). SNPs can be exploited as markers in the search for genes involved in diseases that might respond to drugs (a science called Pharmacogenomics). SNPs are also of major value in Pharmacogenetics, an important area of genomic analysis that is expected to have a major effect on future medical treatment. This science was founded on observations that patients show variable responses to drug treatments. The importance of this can be exemplified by the fact that adverse drug reactions are the sixth leading cause of death in the United States, killing more than 100,000 people annually, with the associated morbidity and mortality costing more than $75 billion annually.

The variability in drug-response is in part due to the genetic variability between patients, including deletions, insertions, variable number of repeat sequences and also SNPs in the patient’s DNA which can thereby be indicators of how well a patient will respond to a drug. In the future it will be possible to use such genetic information to stratify patient populations in preparation for clinical trials such that the difference in drug-response of the various patient groups is more clear-cut. This will reduce the time and cost of clinical trials and,
when linked to diagnostic tests based on the same genotype, ensure that the right patient receives the right drug at the right dose.

Consequently, the high prevalence of SNPs throughout the genome requires efficient methods to analyze these sequence variants. Methods for SNP analysis are in practice limited either to the time-consuming conventional Sanger sequencing that employs separation of reaction products in a slab-gel or capillary, or methods based on sequencing-by-synthesis, with pyrosequencing as the most clear example, and the only one as yet commercialized.

Pyrosequencing is a method in which a cascade of enzymatic reactions generates detectable light. At present, the standard instrument for pyrosequencing is microtiter-based (closed volume chambers) and allows sequence determination of 30-40 bases (Figure 10). However, there are some inherent limitations with this technique, which arise when larger amounts of bases are to be sequenced. Product accumulation, enzyme impurities and decreased enzyme activities could explain these limitations. Another drawback of the present pyrosequencing method is that the reagents are expensive, hence the cost per sequenced base is not competitive with other techniques such as capillary electrophoresis. To address some of the above mentioned problems pyrosequencing was performed in the filter-chamber array presented above [7].

![Figure 10. (a) PSQ™ 96 System dedicated for SNP analysis from Pyrosequencing (b) microtiter plates that are used as analysis vessels.](image)

Two SNPs located on chromosome 17p (codon 72 of the p53 gene) and chromosome 9q (wiaf 1764) have initially been analyzed using pyrosequencing in the filter-chamber device. The coding SNP at codon 72 involves either a G or C residue corresponding to amino acids proline (CCC) or arginine (CGC). These two variants were analyzed several independent times in a device with a 12.5 nl reaction chamber volume. A snapshot of a pyrosequencing reaction in the filter-chamber is showed in Figure 11.
Figure 11 (a) A snapshot of the sequencing reaction where the reaction chamber has the highest intensity (red square).

Figure 11 (b) After completion of the reaction an image was taken to precisely locate the pixels constituting the reaction chamber, inlet, outlet and the alignment mark (right).

In Figure 12, the total amount of collected light is plotted versus time for the pyrosequencing reaction. Extension of the match primer resulted in 5 times more light compared to the mismatch extension.

Figure 12. Total amount of collected light plotted versus time for the pyrosequencing reaction.

We thus proved that it is possible to analyze SNPs in the filter-chamber device. This results in a reduction of sample and reagent usage by a factor of at least 4000 (potentially 100 000). Advantageous features of the microfluidic device compared to the standard instrument are:

- Reduction in size, i.e. it is possible to achieve a portable device
- Reduction in sample and reagent usage, i.e. lower cost/sequenced base
- Enabling flow-through, i.e. less by-product accumulation
- Possibility to integrate sample preparation into the device, i.e. a higher degree of automation
- Enabling of longer read-lengths which opens up for new applications for pyrosequencing

**Protein Analysis**

As a consequence of the successful sequencing of the human genome, scientists today have access to a catalog of all human genes and similar knowledge regarding other organisms, including animals, plants and microorganisms, which is continuously updated. Vast scientific resources will now focus on investigating the
proteins expressed by many of these genes - especially proteins involved in human diseases - in order to accelerate the development of novel biopharmaceutical and small-molecule therapeutics, as well as diagnostic products. The endeavor to characterize and assign functionality to the multitude of proteins encoded in human, animal, plant and microorganism genomes will be one of the largest challenges in biotechnology during coming decades and will require robust, high-throughput methods and reagents to succeed.

Novel ultrahigh-throughput devices will be needed for performing protein analyses that will bridge the technology gap in the discovery process for the biological and life sciences industry. The filter-chamber array presented above has the potential to become an important tool for protein analysis. Experiments have been initiated and some promising results have been achieved.

Asymmetric Catalysis

Asymmetric catalysis is a powerful technique used e.g. by the pharmaceutical industry in the search for new active compounds for pharmaceuticals and other biologically active compounds. The 2001 Nobel Prize in Chemistry will be shared by three scientists who devised techniques for catalytic asymmetric synthesis - the use of chiral catalysts to accelerate the production of single-enantiomer compounds for pharmaceutical use and a wide range of other applications.

Many molecules appear in two forms that mirror each other – just as our hands mirror each other. Such molecules are called chiral, see Figure 13. In nature one of these forms is often dominant, so in our cells one of these mirror images of a molecule fits "like a glove", in contrast to the other one which may even be harmful. Pharmaceutical products often consist of chiral molecules, and the difference between the two forms can be a matter of life and death – as was the case, for example, in the thalidomide disaster in the 1960s. That is why it is vital to be able to produce the two chiral forms separately.

![Figure 13](image)

*Figure 13. Chiral molecules are mirror images of each other.*

High throughput screening for the discovery of new chemical compounds and processes is currently attracting much attention. The purpose of the screening is either to optimize the selectivity and/or reactivity of a chemical reaction or to optimize the properties of a product. Miniaturized systems, like the filter-chamber device, offer many advantages in the field of high throughput screening. A microfluidic device for high throughput screening would reduce the catalyst and reagent consumption and facilitate the separation and control of the end product since the catalyst or reagent is immobilized on the chip surface. Miniaturized catalytic devices can also be used to conveniently gain kinetic and thermodynamic data for reactions. In addition, such devices are suitable for recycling and are therefore environmentally friendly. The filter-chamber device is currently being evaluated for its suitability in asymmetric catalysis and has shown promising results [8].
General Market Considerations

Point-of-care diagnostics is beginning to get noticed. Helped by landmark technologies and the need to contain burgeoning healthcare costs in Europe, the once small sector of the billion-dollar medical diagnostics business is moving to the forefront of many a corporate strategy.

For decades, the diagnostics industry has remained the younger sibling of its pharmaceutical elders. The $20 billion diagnostics industry dominated by Roche, Abbott, Johnson&Johnson and Bayer, has largely been focussed at the hospital laboratory market which accounts for approximately 75% of 1999 market. A report published indicates one of the new and growing areas. The point-of-care (POC) market, with POC being a relatively new phenomenon, made up just over 5% of the 1996 global market revenues.

By far the biggest criticisms against POC tests were the cost per test and robustness. Both are cornerstones of the medical profession and any shortcomings would have doctors reaching for the Drug tariff book. Not surprisingly new technologies are forcing the issue. The emergence of chip-based platforms has raised the awareness of POC detection possibilities. Top of the list comes the DNA chip technology platforms. A common thread weaves through the products; low cost, fast results and central laboratory-level sensitivity. Also on offer is the previously unrivalled access near the patient bedside, in the physicians’ office laboratory or in disaster areas.

The demand for POC solutions will continue as long as technology allows progress. But it is difficult to predict market related forecasts for this segment of the diagnostics industry. No doubt the market value will rise beyond the current 20-25% of total diagnostics, but just how far will it grow is difficult to assess.

Specific Market Expectations

The diagnostic testing is a US $20 billion market worldwide, of which 70-80% is currently conducted in hospital based or commercial laboratories. This type of testing typically involves skilled technicians performing multi-step protocols using sophisticated instrumentation. The remaining 20-25% of the market consists of point-of-care testing. While growth in the laboratory testing market is slow, the point-of-care sector is growing at a rate of 27% per year and is projected to continue in this fashion. This demand is attributed to the drive for cost containment in the health care industry. A conservative estimate of the turnover for the invention presented in this report is about US $20 million.

References


