Microfluidic devices for biomolecular and cellular analysis

Ari Hokkanen
Microfluidic devices for biomolecular and cellular analysis

Ari Hokkanen

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Abstract

In this thesis microfluidic chips were developed for tissue sampling, chemical separation and electrical cell stimulation. Several fluorescence measurement systems were developed in order to enable small volume measurements in microchannels.

Microneedle sampling was developed for cancer tissue analysis. A novel two channel silicon microneedle structure was designed and fabricated. Silicon chip was glue bonded to polymer microfluidic part, which had microfluidic channels as fluid reservoirs and actuation membranes for a fluidic control. Phosphatidylycerine (PC) and phosphoethanolamine (PE) lipids were successfully extracted as biomarkers for breast cancer.

Microchip capillary electrophoresis (CE) was shown on silicon-glass chip, which had integrated thin film electrodes for high voltages (HV). This silicon-glass microchip was used for testosterone measurements with LIF detection. Another CE chip was made by hot embossing PMMA (polymethyl methacrylate). It was used to an antibiotic resistance measurement with mecA gene. Confocal four-colour laser induced fluorescence detection system was developed for roll-to-roll hot embossed PMMA chip.

Glass chip was developed for human embryonic stem cells stimulation. Titanium thin film electrodes were deposited on glass surface. The native beat cycle of embryonic stem cells could be altered from about 1 s to 800 ms with electrical stimulation.

Microfluidics makes fast healthcare analysis possible in doctor’s office or even in home without time-consuming laboratory analysis. Treatment and medication could be started immediately without delay also avoiding unnecessary medication. Microfluidic devices require small sample amounts and low reagent consumption, which can lower cost of diagnosis; and tightly localized diagnosis and treatment, even at single cell level, enabling novel treatments. This is expected to bring major savings for healthcare system in future.

Keywords Microfluidics, microneedle, cell chip, capillary electrophoresis, fluorescence

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Mikrofluidistikkalaitteita biomolekyylien ja solujen analysointiin

Julkaisija
Kemian teknikka korkeakoulu

Yksikkö
Maatalous- ja metsästystekniikan laitos

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Kieli
Englanti

Monografia
X Artikkeliä

Essee

Tiivistelmä

Tässä väitöstyössä kehitettiin syöpäkudusleikkeiden analysointiin mikroneulanäytteennotoa, johon suunniteltiin ja valmistettiin uusi kaksikanavainen piimikroneula. Mikroneulanäytteen nestesäiliöksi ja fluidistikan hallintaan kehitettiin polymeerikanavisto, johon piimikroneula liitettiin liimaamalla. Mikroneulanäytteenoton toimivuus osoitettiin uutamalla phosphatidylycerine (PC) ja phosphoethanolamine (PE) lipidejä, jotka toimivat biomarkereina rintasyövällä.

Kapillaarielektroforeesiin perustuvan molekyylin erotuksen kehitettiin pili- lasimikrokanavisto, jolle kasvatettiin amorfisesta píistä elektrodit sähkökentälle. Tätä pili- lasimikrokanavistoa käytettiin testosteronimittauksiin. Kapillaarielektroforesiin liitettiin myös kuumanpuristettu polymethyl methacrylate (PMMA) kanavisto antibioottiresistanssin mitaamiseen meCA geenin avulla. Tälle PMMA kanavistolle rakennettiin fluoresenssimittausysteemi, jossa käytettiin laserin perustuvaa fluoresenssvirritystä ja konfokalistista nelivärimitattua.

Lisäksi väitöstyössä kasvatettiin lasialustalle titaanielektrodea, joita käytettiin sydänkansan solujen sähköiseen stimulaatioon. Sydänkansasolujen löyntitaituutta pystyttiin muuttamaan sähköisellä stimulaatiolla 1 sekunnista 0,8 sekuntiin.


Avainsanat
Mikrofluidistikka, mikroneula, solu näytealusta, kapillaarielektroforeesi, fluoresenssi

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I would like to thank all the people who have done this work with me. First I will thank Professor Sami Franssila. He was previously a research partner and a co-author in microfluidic research. Now he has been an excellent supervisor of my thesis. He made also possible to write this thesis at Aalto University. Thanks also for peoples in Material science laboratory. This has been great environment for writing of this thesis.

Thank you also for Microelectronics’ people at VTT. It was great place to do research work. Thanks for research managers Jouni Heleskivi, Ilkka Suni, Hannu Kattelus and Philippe Monnoyer. Special thanks for my former group leader Professor Matti Leppihalme. He has always been interested when I will do my thesis. We have regularly discussed about this topic even he retired about ten years ago.

Two former VTT colleagues has also always encouraged me to do my thesis. Thanks to Simo Tammela and Pauli Kiiveri, who hired me to VTT to work with a capillary fiber fluorescence sensor in 1994.

Many thanks for VTT’s microfluidic team at Micronova: Päivi Heimala, Ingmar Stuns, Kai Kolari, Tahvo Havia and team leader Anna Rissanen. You made my thesis possible! We were part of Photonics team for a long time. Thank you for team leader Timo Aalto, Markku Kapulainen, Mikko Harjanne, Sami Ylinen and Matteo Cherchi. MEMS team experience was needed many times for microfluidic development. Thanks especially for Gao Feng about microneedle development.

We needed always collaborators for microfluidics research especially from biotech side. Professor Hans Söderlund developed already capillary fiber sensor with us and his idea was to use the silicon microneedle for a cancer tissue sampling. Kristiina Takkinen, Tarja Nevanen and Harri Siitari developed DNA amplification and capillary electrophoresis with us. Heli Sirén, Stella Rovio, Lotta
Amundsen and Mikko Pakanen also developed capillary electrophoresis with us. Thank you for you all and many others from the biotech side.

Silicon-polymer microfluidics was one of our research topics. Especially the silicon microneedle needs an interface to larger fluidics. Thanks for Oulu peoples, team leader Leena Hakalahti, Ralp Liedert, Annukka Kokkonen and many others.

Electrical cell measurements was an important research topic for our microfluidic team. Professor Jouko Viitanen from Tampere led this electrical cell chip development. Thank you Jouko and other cell research colleagues from Tampere.

Thank you also for all our foreign collaborators. In this thesis Alexander Steinnecker and Philipp Schmid from CSEM developed the robotic system for the microneedle. Jan Budczies from Charité delivered breast cancer tissue samples for the microneedle sampling. Thanks Alexander, Philipp and Jan. Richard Mathies and James Scherer from UC Berkeley developed fluorescence detection with us. Thanks Richard and James.

Finally, I want to thank my family. It is great to have something else than work and studying. This year has been a special for our family, because we have had four students at the same time. Thanks Elisa, Venla, Hannes and Minna.

Espoo, November 2015

Ari
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<tr>
<td>µTAS</td>
<td>micro total analysis system</td>
</tr>
<tr>
<td>AFM</td>
<td>atomic force microscope</td>
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<tr>
<td>APC</td>
<td>allophycocyanin</td>
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<td>APD</td>
<td>avalanche photodiode</td>
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<tr>
<td>bp</td>
<td>base pair</td>
</tr>
<tr>
<td>CAGR</td>
<td>compound annual growth rate</td>
</tr>
<tr>
<td>CCD</td>
<td>charge coupled device</td>
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<tr>
<td>CHO</td>
<td>Chinese hamster ovary</td>
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<tr>
<td>CE</td>
<td>capillary electrophoresis</td>
</tr>
<tr>
<td>CMOS</td>
<td>complementary metal oxide semiconductor</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
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<tr>
<td>CsCl</td>
<td>cesium chloride</td>
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<tr>
<td>CTC</td>
<td>circulating tumour cell</td>
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<tr>
<td>CVD</td>
<td>chemical vapour deposition</td>
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<tr>
<td>DEP</td>
<td>dielectrophoresis</td>
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<tr>
<td>DMF</td>
<td>digital microfluidics</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<tr>
<td>DRIE</td>
<td>deep reactive ion etching</td>
</tr>
<tr>
<td>ECD</td>
<td>electrochemical detection</td>
</tr>
<tr>
<td>EMCCD</td>
<td>electron multiplying charge coupled device</td>
</tr>
<tr>
<td>EOS</td>
<td>electrolyte-oxide-semiconductor</td>
</tr>
<tr>
<td>EWOD</td>
<td>electro-wetting-on-dielectric</td>
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<tr>
<td>FACS</td>
<td>fluorescence activated cell sorter</td>
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<td>FEM</td>
<td>finite element method</td>
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<tr>
<td>FET</td>
<td>field-effect transistor</td>
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<tr>
<td>FFA</td>
<td>free flow acoustophoresis</td>
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<tr>
<td>FITCH</td>
<td>fluorescein isothiocyanate</td>
</tr>
<tr>
<td>FRET</td>
<td>Förster resonance energy</td>
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<tr>
<td>GMO</td>
<td>genetically modified organisms</td>
</tr>
<tr>
<td>HAS</td>
<td>human serum albumin</td>
</tr>
<tr>
<td>HEPA</td>
<td>high efficiency particulate air</td>
</tr>
<tr>
<td>HTS</td>
<td>high throughput screening</td>
</tr>
<tr>
<td>HeLa</td>
<td>human cervical cancer</td>
</tr>
<tr>
<td>hESC</td>
<td>human embryonic stem cell</td>
</tr>
<tr>
<td>HL60</td>
<td>human promyelocytic leukemia</td>
</tr>
<tr>
<td>HV</td>
<td>high voltage</td>
</tr>
<tr>
<td>IDT</td>
<td>interdigital transducers</td>
</tr>
<tr>
<td>IFC</td>
<td>integrated fluidic circuits</td>
</tr>
<tr>
<td>ITP</td>
<td>isotachophoresis</td>
</tr>
<tr>
<td>KCl</td>
<td>potassium chloride</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
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<tr>
<td>Lab on chip</td>
<td>laboratory on chip</td>
</tr>
<tr>
<td>LC</td>
<td>liquid chromatography</td>
</tr>
<tr>
<td>LED</td>
<td>light emitting diode</td>
</tr>
<tr>
<td>LIC</td>
<td>lab-in-a-cell</td>
</tr>
<tr>
<td>LIF</td>
<td>laser induced fluorescence</td>
</tr>
<tr>
<td>LOC</td>
<td>laboratory on chip</td>
</tr>
<tr>
<td>LPCVD</td>
<td>low-temperature chemical vapour deposition</td>
</tr>
<tr>
<td>LTO</td>
<td>low temperature oxide</td>
</tr>
<tr>
<td>MCV</td>
<td>mean cell volume</td>
</tr>
<tr>
<td>MEA</td>
<td>microelectrode array</td>
</tr>
<tr>
<td>MEMS</td>
<td>micro electro mechanical systems</td>
</tr>
<tr>
<td>MFP</td>
<td>microfluidic probe</td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometer</td>
</tr>
<tr>
<td>MTBE</td>
<td>methyl tert-butyl ether</td>
</tr>
<tr>
<td>NSOM</td>
<td>near-field scanning optical microscopy</td>
</tr>
<tr>
<td>OTS</td>
<td>octadecyltrichlorosilane</td>
</tr>
<tr>
<td>PC</td>
<td>phosphatidylcholine</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PDMS</td>
<td>polydimethylsiloxane</td>
</tr>
<tr>
<td>PE</td>
<td>phosphoethanolamine</td>
</tr>
<tr>
<td>PECVD</td>
<td>plasma enhanced</td>
</tr>
<tr>
<td>PEG</td>
<td>polyethylene glycol</td>
</tr>
<tr>
<td>PGA</td>
<td>polyglycolic acid</td>
</tr>
<tr>
<td>PLA</td>
<td>polylactic acid</td>
</tr>
<tr>
<td>PMMA</td>
<td>polymethyl methacrylate</td>
</tr>
<tr>
<td>PMT</td>
<td>photomultiplier tube</td>
</tr>
<tr>
<td>POC</td>
<td>point-of-care</td>
</tr>
<tr>
<td>PPy</td>
<td>polypyrrole</td>
</tr>
<tr>
<td>PVP</td>
<td>polyvinylpyrrolidone</td>
</tr>
<tr>
<td>R2R</td>
<td>roll-to-roll</td>
</tr>
<tr>
<td>RBDW</td>
<td>red blood cell distribution width</td>
</tr>
<tr>
<td>RBL</td>
<td>rat basophilic leukemia</td>
</tr>
<tr>
<td>RIE</td>
<td>reactive ion etching</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>SAM</td>
<td>self-assembled monolayer</td>
</tr>
<tr>
<td>SAW</td>
<td>surface acoustic wave</td>
</tr>
<tr>
<td>Si</td>
<td>silicon</td>
</tr>
<tr>
<td>SiN</td>
<td>silicon nitride</td>
</tr>
<tr>
<td>SiO2</td>
<td>silicon dioxide</td>
</tr>
<tr>
<td>SOI</td>
<td>silicon on insulator</td>
</tr>
<tr>
<td>SPE</td>
<td>solid phase extraction</td>
</tr>
<tr>
<td>Ta2O5</td>
<td>tantalum pentoxide</td>
</tr>
<tr>
<td>TEOS</td>
<td>tetraethylorthosilicate</td>
</tr>
<tr>
<td>TiO2</td>
<td>titanium dioxide</td>
</tr>
<tr>
<td>TiN</td>
<td>titanium nitride</td>
</tr>
<tr>
<td>TPU</td>
<td>thermoplastic polyurethane</td>
</tr>
<tr>
<td>UV</td>
<td>ultra violet</td>
</tr>
<tr>
<td>ZE</td>
<td>zone electrophoresis</td>
</tr>
</tbody>
</table>
This doctoral dissertation consists of a summary and of the following publications which are referred to in the text by their numerals


Author’s Contribution

**Publication I:** Automated microneedle sampling for breast cancer tissue biopsies.

The author did microneedle design, fluidic modelling for silicon microneedle with Comsol Multiphysics, microneedle sampling experiments and wrote the article together with the co-authors. Ingmar Stuns did microfluidic instrumentation and microneedle sampling experiments together with the author. Philipp Schmid built robotic system for microneedle sampling. Annukka Kokkonen developed polymer microfluidic chip. Gao Feng did the cleanroom fabrication of the microneedle. Alexander Steinecker took part in robotic system development. Jan Budczies prepared cancer tissue samples.

**Publication II:** Disposable roll-to-roll hot embossed electrophoresis chip for detection of antibiotic resistance gene mecA in bacteria.

The author carried out fluorescence detection system development and wrote corresponding part of the article. Ralph Liedert did polymer chip development. Lotta Amundsen made CE measurements together with Mikko Pakanen. Minna Mäki and Anne Aittokorpi prepared mecA gene samples. James R. Scherer and Richard A. Mathies took part in fluorescence detection system development.

**Publication III:** Stimulation of human embryonic stem cell-derived cardiomyocytes on thin-film microelectrodes.

The author took part in microchip fabrication masks design with AutoCAD, glass chip fabrication process development and article writing for chip-related parts. Jouko Viitanen created the concept for the cell stimulation with microelectrodes. Päivi Heimala took part in fabrication process development. Kristiina Iljin and Erja Kerkelä took part in cell measurements. Kai Kolari fabricated the glass microchip.
**Publication IV:** Silicon–glass instrumented solid-phase extraction–zone electrophoresis microchip with thin amorphous silicon film electrodes: performance in immunoaffinity analysis.

The author performed microfluidic silicon-glass chip design with AutoCAD. The same design was used in publication V for SU-8 chips. The author wrote the article together with the co-authors. Lotta Amundsen did CE-measurements. Kai Kolari fabricated the chips. Ingmar Stuns did microfluidic instrumentation.

**Publication V:** Design and fabrication of integrated solid-phase extraction–zone electrophoresis microchip.

The author made microfluidic chip design for SU-8 chips. The same design was used in publication IV for silicon-glass chips. The author also did microfluidic and electrical field modelling with Femlab, fluorescence detection system development; and took part article writing. Fluorescence measurement system was modified from publication VI and it was later used in publication IV. Santeri Tuomikoski developed the SU-8 fabrication process. Nina Virkkala and Stella Rovio made CE-measurements.

**Publication VI:** Isotachophoresis of β-blockers in a capillary and on a poly(methyl methacrylate) chip.

The author did fluorescence detection system development, took part in fluorescence measurements and wrote corresponding part of the article. Fluorescence detection system was later used and modified in publications V and IV. Pirkko Kriikku and Benedikt Graß did CE-measurements. Ingmar Stuns built conductivity detector and made microfluidic instrumentation.
1. Microfluidics

The area of microfluidics is concerned with the handling of small volumes of liquid or gas, which in this context ranges from a few μL down to picoliters. This small liquid volume presents challenges in itself as evaporation tends to be fast in open space, therefore closed systems are usually preferred. In fluid mechanics dimensionless Reynolds number is used extensively. It is defined as the ratio of inertial forces to viscous forces. In microfluidics viscous forces are typically dominant and Reynolds number is low. This means that surface forces dominate bulk forces like gravity. In addition, surface tension produces droplets and liquid moves by capillary force. Liquid flow has a laminar flow structure, where parallel lamellae flow without lateral mixing. Liquid mixing is challenging, because it is based on diffusion. On the plus side diffusion is fast in small microfluidic structures, which leads to fast reaction times that are beneficial in many applications.

Microfluidics is still primarily device development, but the number of application-related publications is growing. Figure 1 outlines the number of microfluidic publications since the year 2000 – broken down by scientific field - and clearly shows main application areas. Cell biology and biology are generic, because they overlap almost all other fields.

Figure 1. a) Number of microfluidic related journal papers published in the following categories since 2000: Engineering, Multidisciplinary, Biology and Medicine. b) Word cloud of application areas. The font size is proportional of publication numbers at the application area. Cell biology is exception, it should be five times larger. From [Sackmann 2014].
Small liquid volume pumping and valving development started in the 1970s. In the beginning, the main drivers for microfluidic pump development was their application in insulin/drug dispensing as both require $\mu$L range pumping volumes and small flow rate that can be easily achieved with microfluidic pumps [Woias 2005] [Dumont-Fillon 2014]. The implantable micropump, based on piezoelectric disk benders, was presented in 1975 by Thomas and Bessman [Thomas 1975]. In the 1980s, silicon micromachined microfluidic valves and pumps were developed in Stanford by Smits [Smits 1989][Gravesen 1993]. These pumps were further developed, but published before at Twente by Lintel et al. [Lintel 1988]. Smits had three silicon silicon valves, which all had piezoelectric actuators and it worked as peristaltic pump. Lintel et al. had two or three silicon valves with one piezoelectric actuator on a second valve. These silicon devices are sometimes also known as Micro-Electro-Mechanical Systems (MEMS) type valves and pumps [Woias 2005], because they include mechanical moving membranes for valving and pumping. Polymer devices has been also demonstrated. Quake et al. presented polydimethylsiloxane (PDMS) valves and pumps with multilayer lithography [Unger 2000]. They used later these valves for microfluidic large-scale integration, where they had 1000 chambers with 3574 valves on the same chip [Thorsen 2002]. Diaphragm displacement pumps are a common structure of micropumps and Figure 2 schematically outlines a diaphragm micropump with two valves. The diaphragm provides the actuation and the valves define the direction of liquid movement from inlet valve to outlet valve. The source of diaphragm actuation can utilize many different mechanisms including electrostatic, piezoelectric, pneumatic, thermo-pneumatic and electromagnetic [Iverson 2008].

![Figure 2. Diaphragm displacement pump structure and operation: a) undeflected diaphragm, b) under pressure for inlet valve and c) over pressure for outlet valve. Redrawn from [Iverson 2008].](image)

The first widely used microfluidic application was the inkjet printer head that was developed by IBM in 1977 and featured a silicon based printing nozzle [Bassous 1977]. This breakthrough was subsequently followed by further commercial digital inkjet printer developments in the 1980s by several companies like
Canon, Epson, Hewlett-Packard and Lexmark (IBM spin-off). Today inkjet printing is still one of the biggest applications of microfluidics as the printer heads are required to dispense small pL range droplets on paper. This is made possible by the advances in thermal or piezoelectric actuation coupled to microchannels that allow small ink droplets to be dispensed from big mL range cartridge to the paper.

The first silicon microchannels were developed at Stanford University by Terry et al. and were used in gas chromatography [Terry 1979]. The chip contained a miniaturized injection valve, capillary column and thermal conductivity detector on silicon. Similar microchannels were later developed for other fluidic applications.

The name microfluidics appeared in 1986, when a microchannel for fluidic application was presented by Zdeblick et al. [Zdeblick 1988]. Their paper detailed the development of a microminiature fluidic amplifier on silicon that possessed one input port and two output ports for fluid. The amplifier also featured two gas flows from the side to control fluidic coupling and it was possible to change fluid output with a small gas pressure change.

Micro Total Analysis System (μTAS) name was introduced in 1990 by Andreas Manz who conceptualized that all fluidic laboratory analysis steps could be miniaturized onto the same microchip, with the benefit of a small sample volume and fast, simple and cheap analysis [Manz 1990]. “Fast” means that reaction and diffusion times are short in a small size and in a small sample volume. “Simple” means that professional staff is not needed for analysis. “Cheap” means that reagent and material costs are lower than in laboratory analysis. This initiated microfluidic chip development for numerous applications including analytical chemistry, healthcare, medical and environmental applications [Whiteside 2006]. World’s leading microfluidics conference “μTAS” started in 1994.

New name Laboratory on a chip (Lab on chip or LOC) where mobile miniaturized analysis could take place outside the laboratory was outlined in 1992 by Harrison and Manz [Harrison 1992]. This work demonstrated microchip-based capillary electrophoresis and highlighted that microfabrication should facilitate couplings of capillary systems with “minimum dead volume”. Lab on chip also means integration of many different fluidic components on the same chip, similar to microelectronic chips [Abgrall 2007]. The first microfluidic journal started in 2001 with name “Lab on a Chip” and is now the leading journal in microfluidic research area.

First μTAS/Lab on chip visions were already shown in the 1990s. For example, Burns et al. presented Lab on a chip that included all the required DNA analysis steps from sample loading to gel electrophoresis detection (Figure 3) [Burns 1998]. The added advantage of such a system is that sample and analysis reagent volumes are also significantly smaller as an analysis laboratory is not required.
Moreover, Lab on chip or μTAS systems are eminently portable allowing it to be used to make measurements in the field or in the doctor’s office. The simplicity of such systems also means very little training is required to be able to take samples and make analysis. Overall, this means the potential for extensive money and time savings in areas as diverse as healthcare, environmental measurements or industrial processes.

**Figure 3.** Lab on chip for DNA analysis [Burns 1998].

Single cell measurement in Lab on chip devices has been also demonstrated. Le Gac and van den Berg proposed new name “lab-in-a-cell” (LIC) for single cell measurements in 2010 [LeGac 2010]. It means that a single cell is an experimental unit. Single cell measurements have been also done without Lab on chips, but microfluidics offers parallel single cell analysis. Van den Brink *et al.* demonstrated an array of 16-32 lateral pockets for single cell analysis on a polydimethylsiloxane (PDMS) chip [Brink 2011]. Yobas *et al.* developed microfluidic patch clamp system on glass chip, which could measure 1152 parallel cells at the same time [Tang 2010].

Another term that is also used in this area is biochip. Although biochips can be used for some of the same applications as a microfluidic chip, the biochip does not necessarily have any fluidic structures at all. For example, a biochip could just have a set of electrical measurement electrodes on a planar surface that allow electrochemical measurements to be performed on the chip without the need for microfluidic structures. In addition, microarrays can also be referred to as biochips, for example, a DNA microarray has thousands or millions sample spots on a glass slide surface. These common DNA microarrays do not have microfluidic structures so when the liquid sample is spotted on the surface during a measurement, it stays in position. Protein and cell microarray biochips have been also demonstrated. Those biochips are also without any fluidic structures.
Optofluidics combines the areas of optics and microfluidics and involves the integration of optical lenses into microfluidic devices. This is highlighted in the work by Seo and Lee who integrated lenses into a polydimethylsiloxane (PDMS) chip in order to increase the signal-to-noise ratio of fluorescence detection and make optical alignment much easier [Seo 2004]. It is also possible to utilize optical forces - based on the exchange of momentum between the photons and the irradiated microparticle - in microfluidic systems. The force for the irradiated microparticle is opposite to the change of the photon momentum and such optical forces can be used for microparticle manipulation, sorting and analysis [Jonas 2008]. Optical manipulation has the additional feature that it is a non-contact method, which minimizes the potential for sample contamination. Low force is a problem for optical manipulation, because drag forces overcome easily optical forces, which are in the pN to nN range.

Microfluidics can also be utilized to make optical components as demonstrated by Stephen Gray, who used a liquid droplet as a lens already in 1697 [Gray 1697]. Surface tension causes droplets to adopt a spherical shape, which can then subsequently act as a lens. The addition of microfluidics makes tunable optical components possible without any moving parts, with the tunability provided by geometry or refractive index changes [Levy 2008]. Varioptics [Varioptics] has developed commercial geometry change-based liquid lenses with tunable focus. This is achieved by bending the interface between two immiscible liquids, which allows autofocus and optical image stabilization with electrowetting based actuation [Berge 2000]. Hong et al. have also developed a micro-lens array that can tune the focus via variable refractive index liquid and the attenuation via a dye-containing liquid [Hong 2006].

Another potential application for the field of microfluidics is that of microfluidic cooling for electronics. IBM, in particular, has published several papers related to microfluidic cooling [Colgan 2006]. Microfluidics offer the possibility for more powerful, small and low noise cooling systems when compared to existing heat sinks, heat pipes and fans, as silicon microchannels have the capacity to achieve cooling powers of several hundred W/cm².

Microfluidics has been also used in the area of fuel cells in order to produce smaller and more powerful units. Fuel cells make electricity from chemical energy, like methanol is used as a fuel and catalysts including platinum are often used to enhance reaction rates to ensure that fuel cells offer higher energy density than batteries [Dyer 2002]. It is possible to use microfabrication to produce directly small channel structures that contain catalytic coatings and in this way it is possible to avoid the separate packing process of the catalyte coated beads. Fuel cells also need a fuel cartridge to storing the chemical energy, but recharging is much faster with fuel cells than with batteries [Morse 2007]. Fuel cartridge replacement is almost instantaneous.
There are already a number of commercially available microfluidic products in application areas like diagnostics, point-of-care, medical, environmental and process control that have appeared over the last decade. In general though, the commercialization of microfluidic diagnostics device is challenging, because they are typically low cost products and the revenue stream of one product is typically limited. As a result applications should have high volumes, such that you can make a profitable business, hence the focus is on medical, environmental and process monitoring applications where the unit price can be higher and high volumes are not needed in the beginning. Small device size is important especially in medical applications like drug dispensing and the strength of microfluidics technologies is the ability to integrate several process steps on the same chip. This makes analysis simpler and less time-consuming. Moreover, microfluidics technologies also allow mass manufacture, which makes disposable devices possible. However, application specific development is still needed. That is already happening, because the number of publications is increasing in application related journals [Sackmann 2014].

In 2013 the total microfluidic market size was about $1.59 billion [Volpatti 2014] [Markets and Markets 2013]. The estimated compound annual growth rate (CAGR) of microfluidics is predicted to be 18–29% per year meaning that the microfluidic market will be worth approximately $3-6 billion by 2018 [Markets and Markets 2013] [Yole 2013]. Microfluidics technologies have at least two well recognizable healthcare applications: the glucometer and the pregnancy test [Sackmann 2014]. The glucometer can measure the glucose level from a small blood droplet, whilst the pregnancy test relies on a paper microfluidics based lateral flow assay. These two examples have well-established position on the market and have a big impact on healthcare. They both use small liquid samples, but they do not utilize microfluidic possibilities for fluid handling [Chin 2012]. For example, the lateral flow test although adequate for a pregnancy test, it is not quantitative and thus its utilization in many other applications is limited. Likewise, the glucometer is typically based on electrochemical measurement and there is no sample treatment with microfluidics.

Small volume liquid dispensing is also needed in laboratory analysis and many large laboratory equipment companies like Hamilton, PerkinElmer, ThermoFisher Scientific, Beckman Coulter and Tecan have products in this area. The dispensing heads of such equipment utilizes microfluidic parts like inkjet technology. Shimadzu has, for example, a CHIP-1000 inkjet chemical printer with four piezoelectric printing heads that can produce 87 pL droplets with 55 μm diameter and finds use in protein analysis with MALDI mass spectrometer (MS) [Shimadzu].

Point-of-care (POC) at home and in doctor’s office is one key application area for microfluidics. For example, Abbot has a portable i-STAT equipment for blood analysis that combines fluidics and electrochemical detection to handheld equipment [Abbot]. This machine can do a number of common diagnostic
measurements like cardiac markers (e.g. troponin, B-type natriuretic peptide and creatine-kinase), blood gases, chemistries and electrolytes (e.g. glucose, sodium, potassium, chloride and lactate), coagulation, endocrinology and hematology. Alere also has two microfluidic POC products PIMA CD4 and Triage: PIMA CD4 is for HIV testing and Triage for quantitative BNP, Troponin I, CK-MB, d-dimer, myoglobin, NGAL and qualitative TOX Drug Screen testing [Alere].

1.1 Microfluidic platforms

This chapter outlines the microfluidic platform classification based on five different forces: capillary force, pressure, electrokinetic, centrifugal and acoustic (Figure 4). The platforms mean different types of microfluidic operational principles. Some platforms can produce both continuous and droplet based liquid flows. Of course it is possible to sort these platforms differently and present some alternative classifications as demonstrated in Mark et al. who also identified five microfluidic platforms, but also presented some other platforms like large scale integration, segmented flow and parallel analysis, which are under those five major platforms [Mark 2010].

![Figure 4. Force-based microfluidics platform classification.](image)

### 1.1.1 Capillary force driven microfluidics

Passive fluid pumping is possible for small liquid amounts because liquid will move in small channels by capillary force. Intermolecular forces generate capillary force between the liquid and solid surface. Surface tension of the liquid and adhesion between the liquid and solid surface works against gravity. Capillary force phenomenon was first discovered by Leonardo da Vinci [Unesco 1974]. He noticed liquid capillary movement in paintbrush. Capillary action was later presented as height $h$ of a liquid column

\[
h = \frac{2\gamma \cos \theta}{\rho gr} ,
\]  
\[ (1)\]
where $\gamma$ is liquid-air surface tension, $\theta$ liquid contact angle on solid surface, $\rho$ liquid density, $g$ gravity and $r$ radius of tube [Batchelor 1967]. Capillary flow velocity was defined by Washburn as

$$\frac{dl}{dt} = \frac{r \gamma \cos \theta}{\eta 4l},$$

where $l$ is capillary length, $t$ time, $r$ radius of capillary, $\gamma$ liquid-air surface tension, $\theta$ contact angle and $\eta$ viscosity of liquid [Washburn 1921].

Dimensions of microfluidic capillaries are typically less than few hundreds micrometers and the presence of a hydrophilic surface with low contact angle is needed for capillary action. This usually means usually an oxide coating for silicon microchannels. Microfluidic channels on glass chip are inherently hydrophilic. On the other hand, chemical or plasma treatments are required in polymer microfluidics to make channels hydrophilic [Barbier 2006] [Kitsara 2013]. The channel dimensions and hydrophilic coatings define the typical flow velocity of equation (2) in capillary force driven microfluidics with levels of $>1$ m/s in micrometer scale channels and <1 mm/s in large hundred micrometer channels. Siljegovic et al. utilize this capillary flow velocity in capillary force driven microfluidic networks [Siljegovic 2005]. They made hydrophilic polymer microfluidic channels with CD injection molding and 30 s oxygen plasma treatment. They used capillary flow control and geometrical valves for 1-5 s delays in a microfluidic network.

Passive fluid valving is achieved by using a hydrophobic coating or introducing a channel waist with a geometrical change in the microfluidic channel. The hydrophobic coating stops liquid flow by changing the contact angle of the liquid, whilst the channel waist stops liquid flow, because capillary force is lower after channel waist thus a passive valve can stop liquid flow without any external force. It is typically opened after some wetting time or with external force. Passive valves are usually single use, but they could also be reversible, if they have time to dry before the next sample. Feng et al. tested hydrophobic octadecyltrichlorosilane (OTS) self-assembled monolayers (SAM) and plasma deposited CHF3 layers on hydrophilic silicon dioxide (SiO2) channel, which has Pyrex lid with the same hydrophobic coatings [Feng 2003]. Their first design was based on a geometrical change with unpatterned hydrophobic OTS-SAM layer and the second design on CHF3 hydrophobic patch on all four surfaces of the channel: the floor and the sidewalls were deposited in the first step, and the ceiling in another, followed by bonding. The rest of the channel was SiO2 in both cases.

A C-reactive protein (CRP) test based on passive pumping and valving has been reported by Gervais et al. and is shown schematically in Figure 5 [Gervais 2009]. The capillary pump on the right pumps blood serum from the left through microfluidic channel and the blood serum starts to move with capillary force. When the blood serum reaches the capillary pump, it will start to fill the capillary pump as it has the largest capillary force in the system. The presence of the
flow resistors and the capillary pump determine the flow velocity in the reaction channel. The capillary pump also defines a maximum pumping volume of 5 μL for the system and it takes a few minutes to pump 5 μL sample from blood filter to capillary pump. The CRP test itself has three antibody coatings: First zone (#1 at Figure 5) has detection antibodies for blood serum CRP analytes; Second zone (#2) has capture antibodies for detection antibodies that have bound to analytes so only the detection antibody captured analyte will bind to zone #2. The final zone (#3) is an assay control for the test and excess detection antibodies will bind to the control line antigens.

![Diagram of CRP test](image)

**Figure 5.** CRP test presented by Gervais et al. [Gervais 2009]. Chip has a sample loading pad for filtering serum from blood. Microfluidics part has sample collector, delay lines, flow resistor and capillary pump for fluid flow control. CRP assay has three zones: 1 detection antibodies, 2 capture antibodies and 3 control antigens.

Paper-based microfluidics are also capillary force driven as paper possesses a porous hydrophilic cellulose structure. Müller and Clegg started paper microfluidics in 1949 [Müller 1949]. They made paraffin barrier filter paper and used this device for chromatographic separation. They separated colour pigments in paper channel and used monochromator and photomultiplier tube (PMT) for optical detection. Later ten different fabrication methods has been used for paper based microfluidics: photolithography, plotting with an analogue plotter, ink jet etching, plasma treatment, paper cutting, wax printing, ink jet printing, flexography printing, screen printing and laser treatment [Li 2012]. All these methods produce hydrophobic barriers to hydrophilic paper except paper cutting. Paper microfluidics has both two and three dimensional structures. The narrowest line width 62 μm has been done with treatment [Chitnis 2011]. Simple assays are already possible. Carrilho et al. demonstrated protein, cholesterol and glucose assays [Carrilho 2009].

### 1.1.2 Pressure driven microfluidics

Flow behavior in tube or pipe is described with a dimensionless Reynolds number

\[
Re = \frac{D \nu \rho}{\mu},
\]

where D is the diameter of the pipe, \( \nu \) average velocity, \( \rho \) fluid density and \( \mu \) fluid viscosity [Fellows 2000]. Viscous forces are typically high and Reynolds number low at microfluidics. It is not possible to give exact number for laminar flow, because it depends on a geometry. In pipe flow lower Reynolds numbers than
2100 means laminar flow, 2100-4000 is transition range and over 4000 means turbulent flow. Laminar flow has parabolic flow distribution. There are no lateral flows and liquid mixing happens only by diffusion. Inner wall has zero velocity, because surface forces dominates.

Pressure driven microfluidics is mostly performed via macroscale pumps and valves with pressure, syringe and peristaltic pumps used. Pressure and syringe pumps offer very stable flow velocity. The high pressures in small microfluidic channels are problematic, because pressure drop $\Delta P$ varies $1/r^4$ as expressed by Hagen-Poiseuille equation

$$\Delta P = \frac{8\mu L Q}{\pi r^4},$$

where $\mu$ is viscosity, $L$ pipe length, $Q$ volumetric flow rate and $r$ radius of a channel [Westerhof 2010]. Peristaltic pumps are used when a pulsating flow is allowed, however such pumps have elastic tubing, which restricts their use in high pressure applications.

Pump and valve based microfluidics are based on pressure gradients within the fluidic system [Mark 2010]. This pressure gradient typically creates a stable laminar flow in a microchannel, which can be used for diffusion mixing of different liquids as in Figure 6. Inertial forces can be utilized for particle separation in a laminar flow. This was clearly demonstrated by Hanson et al. who used the inertial forces in a PDMS device comprising of a single inlet and two outlets to filter 10 $\mu$m sized particles with an efficiency 95-97% [Hansson 2012].

![Figure 6. Diffusion mixing for gradient generation with pressure driven laminar flow.](image)

Pinched flow fractionation in microfabricated device was first presented by Yamada et al. and the laminar flow profile they used is shown in Figure 7 [Yamada 2004]. The laminar flow profile in the microchannel is parabolic in input channels, because the shear stress decreases flow velocity on the channel surface to zero. Lower input liquid without particles push particles from upper liquid against the upper wall at the pinched segment. The size of the particle defines the position of the particle. Flow profile then spreads after a pinched
and broadened channel part causing different size particles goes to different direction as their position in the flow profile is different, thus allowing the 15 and 30 μm polystyrene particles to be separated.

![Diagram](image)

**Figure 7.** Pinched flow fraction with green 15 μm and red 30 μm polystyrene particles. Redrawn from [Yamada 2004].

Droplet microfluidics is a new way to do microfluidic analysis [Teh 2008] [Seemann 2012]. Each droplet is a separate sample, which could include molecules, cells, particles or just washing liquid. These droplets could be in air or in some other liquid like oil and the term multiphase microfluidics is also used to describe these oil-embedded systems. Liquids should not be miscible, if it is liquid droplets in a liquid. A droplet could just carry a sample, but it is also possible that whole analysis can be performed in one single droplet. Droplet microfluidics offers a high throughput system, because the sample number can be in the range of millions as it depends on droplet production rate and flow velocity. There are several techniques to generate and handle droplets in droplet microfluidics system, one of which is pressure driven droplet microfluidics that is outlined below.

Pressure driven droplet microfluidics means pump and valve based droplet system in closed channel [Teh 2008] [Seemann 2012]. It has the ability to generate the same size droplets in a row in a microfluidic channel by accurate pumping control in a channel cross where sample liquid and carrier gas/oil are pumped to the same channel. For example, Mazutis *et al.* have sorted cells that were inside pressure driven droplets as shown in Figure 8 [Mazutis 2013]. The droplets were in the oil carrier and sorting was based on the dielectrophoretic force of an electrical field in conjunction with fluorescence detection. Mazutis used “flow focusing” geometry to generate monodisperse droplets [Shelley 2003]. The outer oil flow rate is always larger than the inner water flow rate. The outer oil flow exerts pressure and viscous stresses to force the inner water flow into the same narrow channel, which then breaks water flow to droplets. The droplet diameter is comparable to the channel diameter. A surfactant is needed to avoid uncontrolled coalescence [Mazutis 2013]. Surfactant molecules prevent droplet
coalescence by populating water-oil interface after few milliseconds stabilization time. These surfactants have also some drawbacks, because they interact also with the sample. Diffusion and reaction times may altered.

![Diagram of droplet microfluidics](image)

**Figure 8.** Droplet microfluidics is applied to single cell research. Redrawn from [Mazutis 2013].

Valve-based microfluidics where it is possible to reprogram the route for the liquid has been presented by Jensen et al. [Jensen 2013]. They demonstrated a pneumatic actuation based programmable microfluidic system, where the liquid sample plug was between valves. They called this system as digital microfluidics (DMF), but it should be called large scale integration as in [Mark 2010]. By using different type inlet/outlet, mixing and combining valves were in a matrix format, this valve based large scale integration can carry out the same operations as electric and acoustic droplet DMF systems detailed below.

Pressure driven droplet microfluidic chips are already commercially available from many manufacturers including Dolomite microfluidics [Dolomite] and Micronit [Micronit]. These are droplet generators for research use as they require extra instrumentation and development to work with specific applications. It is also possible to buy complete systems like the RainDrop Digital Polymerase chain reaction (PCR) System from RainDance [RainDance].

1.1.3 **Electrokinetic microfluidics**

As the name suggests liquid movement is controlled by an electrical field in electrokinetic microfluidics. It is possible to pump/mix liquids, separate molecules and move micro particles or cells by electrical field [Mark 2010].

Electrokinetic microfluidics offers a first DMF system, which is based on electrowetting-on-dielectric (EWOD) or on electrostatic force [Choi 2012]. In EWOD the electrical field changes the contact angle of the liquid on one side of the droplet, which in turn generates droplet movement [Fair 2007]. The electrostatic force based version do not need a large change to the contact angle and it is more versatile, because it can move also dielectric liquids and low-surface-tension liquids [Abdelgawad 2009]. Both electrical DMF systems have droplets
on an open/closed dielectric hydrophobic surface: The open device has all electrodes on lower surface, whereas the closed device has a continuous ground electrode on top surface. The electrodes are placed in a fixed array format, but droplet movement is freely programmable so it is possible to mix, combine and separate droplets as well as to select input and output positions for this system. Figure 9 displays a schematic of an electrode array system used for digital microfluidics. Wu et al. have used a EWOD system for droplet generation and reagent mixing in a microfluidic system by utilizing combined reagent sample droplets in continuous droplet flow and separate washing droplets [Wu 2011].

One of the drawbacks with an open DMF device is evaporation, but overall it gives more freedom for a sample input. This has been investigated by Shih et al. who dried blood spots on an electrostatic DMF chip [Shih 2012]. They did sample pretreatment with digital microfluidics and subjected the sample with off-chip injection to MS analysis.

Electrostatic DMF has an open-source automation system which is known as DropBot and that has been developed by Aaron Wheeler et al. [DropBot]. It is possible to buy EWOD digital microfluidic based commercial systems, for example, Illumina’s NeoPrep system for next-generation sequencing platforms that is based on microelectronics rather than the previously used capillary electrophoresis methodology [Illumina].

Electro-osmotic flow was first observed by F.F. Reuss in 1808 [Briggs 1917]. He put two glass tubes into moist clay and filled them with water. Next he inserted electrodes into glass tubes and established a potential gradient between the electrodes. Reuss noticed liquid movement from anode electrode to cathode electrode. In addition, some detached clay particles moved in opposite direction. Electro-osmotic flow is later utilized to pumping, where channel walls are charged and liquid moves in electrical field as it is presented in Figure 10 [Kirby 2009]. Channel walls have an electrical double layer and Coulomb force moves positive ions on the inner wall to the direction of a negative potential, when an electrical field is coupled to the liquid channel. The flow profile of liquid is flat.
within the channel cross-section and thus is different from the typical parabolic laminar flow profile shown in Figure 7. Manz et al. used electro-osmotic flow for sample injection in microchip capillary electrophoresis on a glass chip [Manz 1994]. A flat fluid profile of electro-osmotic flow in Figure 10 offered accurate injection compared to a parabolic profile of pressure driven laminar flow in Figure 7. Schasfoort et al. demonstrated electro-osmotic flow based controlling and switching element in a silicon nitride (SiN) microchannel [Schasfoort 1999]. They presented this as a microfluidic field-effect transistor for a fluidic network.

![Diagram of electro-osmotic flow in capillary channel](image)

Figure 10. Electro-osmotic flow in capillary channel. Redrawn from [Kirby 2009].

Capillary electrophoresis (CE) separates molecules in electrical field as a function of size and charge. CE is discussed more detailed later in this thesis.

Dielectrophoresis (DEP) force moves or traps dielectric particles like cells in an electrical field. The electrical field polarizes the particles, which start to move along the field with movement dependant on the surrounding medium, electrical field and particles' electrical properties, shape and size. Herbert Pohl first described DEP in 1951 [Pohl 1951] and later used the technique to separate living and dead cells [Pohl 1966]. DEP force is presented [Hughes 1998] as

\[ F_{DEP} = 2\pi r^3 \varepsilon_m \text{Re}[K(\omega)] \nabla E^2, \quad (5) \]

where \( r \) is the particle radius, \( \varepsilon_m \) the permittivity of the medium, \( \nabla \) del vector operator, \( E \) electrical field and \( \text{Re}[K(\omega)] \) is the real part of the Clausius-Mossotti factor

\[ K(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}, \quad (6) \]

where \( \varepsilon_m^* \) and \( \varepsilon_p^* \) are the complex permittivities of the medium and particle respectively, and \( \omega \) angular frequency. Complex permittivities are

\[ \varepsilon^* = \varepsilon - j\sigma/\omega, \quad (7) \]
where $\varepsilon$ is permittivity, $j$ imaginary unit and $\sigma$ the conductivity.

Dielectrophoretic trapping was demonstrated by Hughes et al. [Hughes 1998]. They trapped virus particles, capsids and latex spheres in the size 93-250 nm. DEP is also used to manipulate microparticle or cell movement for the purposes of sorting [Yi 2006]. Gascoyne et al. separated cancer cells from blood by exploiting the presence of microelectrodes in microfluidic chambers [Gascoyne 1997]. Platelets have also been purified from diluted whole blood by Pommer et al. who developed DEP-activated cell sorter that managed to get a ~95% purification rate for platelets [Pommer 2008].

Electrokinetic mixing is based on liquid (rather than particle) movement in an electrical field and it does not require any moving parts [Chang 2007]. In contrast, passive electrokinetic mixing can use a DC field and channel/surface structures to enhance mixing. Alternatively, active electrokinetic mixing makes use of an AC field or switched DC field. Active mixing produce repeated stretching and folding without channel and surface structures. Both passive and active mixing can produce chaotic mixing.

1.1.4 Centrifugal microfluidics

Centrifugal spinning can be used to drive fluid flows in microchannels. Volumetric flow rate in centrifugal microfluidic channel is

$$Q = A d_H^2 \frac{\rho \omega^2 \bar{r} \Delta r}{32 \eta L}, \quad (8)$$

where $A$ is cross-sectional area of a channel, $d_H$ hydraulic diameter of a channel, $\rho$ density of a liquid, $\omega$ angular velocity, $\eta$ viscosity of a liquid, $L$ length of a channel [Duffy 1999]. Channels position parameters $\bar{r}$ and $\Delta r$ are

$$\bar{r} = \frac{r_1 + (r_0 - H)}{2}, \quad (9)$$

$$\Delta r = r_1 - (r_0 - H), \quad (10)$$

where $r_0$ and $r_1$ are inner and outer radial positions of the channel, respectively; $H$ is the liquid level in a feed reservoir [Duffy 1999]. Geometrical parameters are presented in Figure 11.

![Figure 11. Schematic presentation of geometrical parameters for volumetric flow rate. Redrawn from [Duffy 1999].](image-url)
Centrifugal microfluidics uses disk manufacturing experience from CD and DVD. It is able to perform all basic operations like pumping, metering, valving, mixing [Ducrée 2007]. Centrifugal force pushes liquid forward in the channel or it opens hydrophobic/geometric valve and liquid flows with capillary force.

The use of intelligent channel designs also makes it possible to fill many parallel reaction chambers with predefined liquid volume. Strohmeier et al. have integrated parallel DNA amplification into centrifugal microfluidics for food-borne pathogens, as outlined in Figure 12 [Strohmeier 2014]. Six parallel 5 μL samples are mixed and amplified. Liquid flows are controlled just by rotating a disk with different frequencies: 0-25 Hz. Figure 12 shows sample preparation before DNA amplification. After fluidic sample preparation, microfluidic disk was moved to Qiagen’s Rotor-Gene 2000 commercial thermal cycler, which had air mediated heating/cooling between 60-95°C and real-time fluorescence detection.

**Figure 12.** Centrifugal microfluidics for DNA analysis from [Strohmeier 2014]. a: PCR mastermix and elution buffer loading with rotation frequency f=8Hz. b: PCR master mix is divided to metering fingers and elution buffer rotated to elution buffer chambers with f=12Hz. c: PCR master mix is dispensed to measurement chambers with higher f=25Hz. d: buffer and DNA are mixed in intermediate chamber with alternating f=0/7Hz. e: buffer-DNA mix is divided to metering fingers with f=18Hz. f: and dispensed to measurement chamber with f=25 Hz.

CD and DVD equipments offers one extra additional feature, because it is possible to utilize optical reader system for detection. Tortajada-Genaro et al. used centrifugal microfluidics and DVD drive for genetically modified organisms (GMO) testing with food samples by [Tortajada-Genaro 2015]. They printed a microarray of DNA primers into centrifugal microfluidic device and made iso-
thermal amplification for the food sample. Centrifugal microfluidics was designed for 500-2000 rpm spinning and it included hydrophobic capillary valves, which were closed under 1500 rpm. They reprogram optical reader with their own software and use DVD’s 650 nm laser for detection. They got detection limit 7 μg/g detection limit that is less than European Union regulation limit.

1.1.5 Acoustic microfluidics

Acoustic force for droplet ejection was presented as early as 1927 by Wood and Loomis who made oil droplets using acoustic force [Wood 1927]. Surface acoustic waves (SAW) are especially applied in area of microfluidics as a SAW can comprise of a sound wave on a material surface [Lin 2012] [Ding 2012] [Ding 2013]. For example, an electrical field can be used to generate a propagating mechanical stress on a piezoelectric surface and this mechanical stress is coupled to liquid and mechanical vibration starts to travel in liquid [Ding 2013]. Travelling acoustic waves can move particles or pump/mix liquids, while standing acoustic waves can capture particles. A travelling acoustic wave needs at least one interdigitated transducer (IDT), whilst standing acoustic waves need at least one pair of IDTs – the interference of the two acoustic waves generates the standing acoustic wave.

Particles and cells have been manipulated by SAW. Shi et al. developed so-called acoustic tweezers for microparticle and cell manipulation [Shi 2009]. They use standing SAW, where particles stay in pressure nodes. They have two orthogonal interdigital transducers for SAW actuation and two liquid connections in the corners of square shaped device. Schematic picture of standing wave SAW device is presented in figure 13.

![Figure 13. Schematic picture of acoustic tweezers for microparticle and cell trapping. Redrawn from [Shi 2009].](image)

It is possible to pump continuous fluid flow by using a travelling SAW and Fallah et al. have made use of this technique for cell adhesion studies [Fallah 2010]. They had a closed loop channel and single SAW actuator which circulated the
liquid sample in a microfluidic channel and measured the level of cell adhesion on biofunctionalized surface.

SAW is also a second option for digital microfluidics, as it is possible to perform the same kind of droplet actuations with SAW as with electrical DMF. Guttenberg et al. have previously demonstrated DNA amplification by manipulating 200 nL oil covered droplets on a piezoelectric LiNbO3 substrate with a SAW. The oil was used to avoid sample evaporation during thermal cycling, which was done between 50-95°C. [Guttenberg 2005].

Axial acoustic waves has been also used for free flow acoustophoresis (FFA) by Petersson et al. [Petersson 2007]. Standing acoustic wave was generated into laminar flow and microparticles were separated based on their size and density. Silicon channel for separation was 370 μm wide and 125 μm deep. FFA was first demonstrated with 2-10 μm polystyrene particles. Next it was shown that it is possible to separate normally inseparable particles by manipulating the density of the suspending medium with cesium chloride (CsCl). Red cells (~7 μm diameter, ~2 μm thick), platelets (2-4 μm diameter) and leykocytes (5-20 μm diameter) were successfully separated by adding 0.22 g/mL CsCl into the suspending medium.

Acoustic droplet ejection has been used to modify inkjet printers and liquid dispensing. EDC Biosystems [EDC] and Labcyte [Labcyte] manufacture acoustic liquid dispensing systems.

### 1.2 Microfluidic applications

This chapter divides microfluidic applications into three main categories: physical, chemical and biological. A couple of examples from each main categories are presented. These examples are selected so that the author has some own contribution to each topic.

Physical applications are based on microfluidic phenomena like pumping, valving, metering, sorting, filtering, extraction, dispensing and spraying. Microneedles and microfluidic filtering are used here as examples of such physical applications. Microneedles and filters are, however, also used in chemical or biological applications. Physical microfabricated structure itself is essential for microneedles and filters for which reason application category is here physical.

Chemical applications concern reaction and/or separation of species in this case microchip capillary electrophoresis and DNA amplification are used here as examples of chemical applications.

Biological applications have living cells or bacteria in microfluidic structures. Cell culturing and electrical cell measurements are used here as examples of biological applications. There are many types of cells like mammalian, bacteria
and yeast cells: Bacteria cells are typically small and robust, while mammalian cells are more sensitive for environmental conditions. This thesis concentrates on mammalian and yeast cell applications. Cell filtering is presented in physical applications with other microfluidic filters.

1.2.1 Physical applications

Microneedles

Handmade small needles have been used for decades and drug delivery microneedles have been designed since the 1970’s [Kim 2012]. The first microfabricated needles were developed in 1990’s initially from silicon, because microfabrication tools were mainly for silicon. Metals and polymers are now more common materials, but glass and ceramics have also been used. In addition, silicon has been also used as a mould material for example, Lutte et al. used a silicon mould for 430 μm long SU-8 microneedles [Lutte 2007] and 560 μm long ceramic microneedles [Bystrova 2011]. Silicon, glass and ceramic microneedles will break easily, if you bend them. Although these materials are strong for perpendicular forces, their bending stiffness is low. In addition, they are biocompatible or at least can be easily coated with biocompatible materials.

In contrast, metals and polymers have a higher bending stiffness and in particular, stainless steel syringe needles have been used for a long time due to their good strength for perpendicular force. Polymer microneedles have also sufficient strength for perpendicular force. Some polymers are biocompatible or even biodegradable materials like polylactic acid (PLA) and polyglycolic acid (PGA) [Park 2005] [DeMuth 2013]. Biodegradable materials increase safety, if microneedle breaks into the skin. They will dissolve and disappear over time.

Microneedles come in four major designs: solid, coated, dissolving and hollow (Figure 14) [Kim 2012]. The first three types are not really microfluidic needles as they do not have any microfluidic channel. Solid microneedles has been used for electrical measurement, drug delivery and skin treatment. In electrical measurements the purpose is painless electrical contact through the skin. Electrical microneedles are also used for implantable brain measurement electrodes. In drug delivery, a solid microneedle is used to make pores, after which the drug is applied to the skin and this methodology can enhance drug delivery by 1–2 orders of magnitude [Park 2010].
The second needle design is drug coated microneedle and this type of microneedle does not need a separate drug dosing phase as the drug coating is dissolved into the skin during the skin permeation. The third needle structure is dissolving microneedle the tip of which is designed to stay inside a skin and to allow the drug to dissolve. Dissolving microneedles typically have a drug in water-soluble needle or in biodegradable polymer. Sullivan et al. fabricated dissolving polyvinylpyrrolidone (PVP) microneedles with photo-polymerization [Sullivan 2010] and coated/dissolving microneedles are used for example in vaccination [Prausnitz 2009].

The fourth design is a hollow needle, which is the only real microfluidic based design and was first used for painless blood sampling and drug injection [Gardeniers 2003] [Griss 2003]. Gardenier et al. combined deep reactive ion etching (DRIE) and wet etching for 150-350 μm long silicon needles. Griss et al. developed 210 μm long side-opened cross-shaped silicon needle with DRIE. The skin structure is presented in Figure 14 and comprises of: the stratum corneum (outer layer) consisting of dead cells; the second layer (<200 μm) is the viable epidermis that has outermost layer of cells, but it does not contain blood vessels or nerve fibers and the third layer, the dermis consists of connective tissue that has blood vessels and nerve fibers. This means that painless blood sampling is quite a challenge because blood vessels and nerve fibers are present in the same layer. In order to achieve this aim, Li et al. developed 1800 μm long nickel microneedles, with a 120 μm tip outer diameter and 15° laser cutted bevel angle with which they were able to extract 20 μL blood sample from tail artery of mouse [Li 2013]. Painless drug injection and vaccination are easier as only injection is needed. Roxhed et al. utilized a hollow 400 μm long silicon microneedle device that comprised of a 12 μL chamber for insulin and actuator for the active infusion of insulin [Roxhed 2008]. They assumed that this microneedle is already painless, because actual penetration depth was much shorter than 400 μm. They tested their system on diabetic rats with a 2 μL/h infusion and
got five times higher insulin concentration in blood plasma after three hours with active pumping when compared to passive diffusion without pumping. Gardeniers and Griss should also have painless needles, because needle lengths were about 200 μm [Gardeniers 2003] [Griss 2003].

In contrast, this thesis details the use of a multiport microneedle for breast cancer diagnostics [Publication I]. The microneedle was used to extract lipids from tissue samples and the lipids were subsequently used as biomarkers for cancer. This microneedle had two versions with 150 μm and 250 μm diameters. Both versions were about 200 μm long. A blunt needle tip was the main difference to other microneedles, which have sharp needle tips for blood sampling or drug delivery.

There are commercial microneedle products available and they are typically used for cosmetic applications. In skin treatment, Dermarollers – which comprise of solid stainless steel microneedles on a roller - make pores into skin surface, after which a cosmetic cold cream is applied to skin [Dermaroller]. CosMED Pharmaceutical Co. Ltd also produces the MicroHyala® dissolving microneedle product that contains hyaluronic acid to combat wrinkles [MicroHyala].

In addition to cosmetic use, there are also microneedle products for drug and vaccine delivery. Becton Dickinson has the Soluvia® hollow steel needle for syringes that comprises of a 30 gauge needle (outer diameter 318 μm) is 1.5 mm long that has been tested for vaccine delivery with a 120 μL fluid volume [Laurent 2007] [Soluvia]. 3M has also both hollow and solid polymer microneedles for drug delivery which are currently in clinical trials [3M]. Li et al. tested 3M’s solid needles with copper peptides, which are also naturally in blood plasma [Li 2015]. Copper peptides are needed for skin regeneration and wound healing. This plastic device had 351 pyramid needles, which were 700 μm long and with 500 μm spacing. NanoPass Technologies Ltd. has got FDA clearance for MicronJet® silicon microneedle product, which has a plastic adapter for syringe use [MicronJet]. The pyramid shape silicon needle is 450 μm long and has been tested for example in influenza vaccination with 100 and 200 μL fluid volume [Damme 2009]. Debiotech also has silicon microneedle system with 350-900 μm long needles that comes either with a special syringe or plastic syringe adapter [Debiotech]. All these commercial microneedles are longer than 200 μm. They will reach the dermis layer of the skin and produce some pain.

**Microfluidic filtering**

Microfluidics makes it possible to do micro- and nanometer particle filtering and sorting. Typical filter structures include membranes, pillars, sacrificial and weir filters [Franssila 2010a]. It is also possible to make the filters without using microfabrication technologies, but microfluidic filters are much easier to integrate as a part of the lab-on-chip configuration. Microfabrication is more accurate in pass size and it has also more precise tuning possibilities than traditional
machining. Traditional cellulose and polymer filters have high porosity values, but they have less tuning possibilities than microfabrication, because the tuning is based solely on the material properties. In microfabrication, size of the hole and pillar spacing is tuned with lithography and by the etching process. For example, the porosity of a sacrificial filter can be tuned by process parameters, whereas the size of a weir filter is tuned with etching time.

The construction of pillar and membrane type cell filters tend to be problematic for glass and polymer materials, but with silicon the accuracy is sufficient. Pillar diameters are typically a few tens of micrometers with heights of less than a few hundred micrometers and pillar spacing is typically > 1 μm. In contrast, membrane filters have holes that range from 0.1 to a few micrometers. Sacrificial filter makes a nanometer size filtering possible and 10 nm is manufacturable from a fabrication point of view [Franssila 2010a]. Sacrificial filter could be generated to holes in silicon. Holes are covered with a conformal thin film e.g. oxide and then closed with polysilicon. Next polysilicon is patterned so that it is possible to remove oxide between silicon and polysilicon. This way nanometer range holes are generated for a sacrificial filter and the filter size is just oxide thickness dependent.

Bead filtering is used to immobilize a desired sample on bead surface, for example, Andersson et al. captured 5.5 μm polystyrene beads and 2.8 μm magnetic beads with silicon pillar filter [Andersson 2000]. Later Russom et al. used the same flow-through device for single-nucleotide polymorphism analysis with fluorescence detection and with pyrosequencing (a bioluminometric DNA sequencing method) [Russom 2003] [Russom 2005]. Additionally, microfluidic filters have been used as a frit for a separation column of liquid chromatography (LC). Lazar et al. for example have developed a microfluidic LC on a glass microchip for proteomics and biomarker screening [Lazar 2006]. In their work they filtered 5 μm (Zorbax, SB-C18, Agilent Technologies) particles with weir type filter, which 7-10 μm wide and 1.5-1.8 μm deep channels. Moreover the author of this thesis has developed a microfluidic filter for sample concentration (Figure 15). Agarose beads were captured in the filter and the sample was concentrated inside these beads with solid phase extraction (SPE). Figure 15 displays the test structure for the SPE and it was later coupled to testosterone detection with microchip capillary electrophoresis [Publication IV] [Publication V].
Figure 15. Author developed silicon pillar filter for agarose beads. Silicon pillars were 50 μm wide, about 350 μm high and they have 30-50 μm spacing (a constant spacing in the same chip).

Microfluidics makes it possible to filter and sort living cells in straightforward fashion. Weir filters however are not necessarily good cell filters as cells are typically soft and flatten. In contrast, the sacrificial filter type is good for bacteria cell applications. Bacteria cells are in the micrometer range, but because they are malleable, they can penetrate through submicron filters.

On the otherhand, pillar and membrane filters are good for human cells or yeast cells, which are typically on the order of 3-20 μm. We have developed a high efficiency particulate air (HEPA) membrane filter based on a silicon on insulator (SOI) wafer [Kolari 2010b]. This filter was used for yeast cells in a bioprocess monitoring application and proved to be suitable for online measurements with regenerative washing steps. Our silicon membrane filter is shown in Figure 16 and featured 750 nm holes in the 8 μm thick SOI membrane, which were subsequently filled down to 350 nm with thermal oxidation.

Figure 16. Microfluidic membrane filter on SOI wafer [Kolari 2010b]. Filter membrane had 350 nm holes with 2.5 μm spacing.

Microfluidic filters are used to sort red and white blood cells, but it is even possible to separate different size red blood cells [Layton 2012]. Blood have three
kinds of cells: red blood cells (4.2-6.1*10^6/μL), white blood cells (4.5-10*10^3/μL) and platelets (150-400*10^3/μL) [MedlinePlus CBC]. These three cell types has 45% of whole blood and the rest 55% is blood plasma. Layton et al. demonstrated a silicon filter gradient for red blood cells that comprised of pillar filter gaps that ranged from 10 to 3 μm with 1 μm steps. Such a filter gradient is not easy to fabricate with conventional filters. Cell sizes are usually measured with a flow cytometer that measure one cell at a time. The microfluidic filter gradient chip is much faster as it involves a parallel measurement for all cells at the same time. The size variation of red blood cells can be calculated and a normal value is 11.5-14.5% [RBDW eMedicine]. A high red blood cell distribution width (RBDW) can be used as a diagnostic of anemia, folate and vitamin B12 deficiency, but it also requires the mean cell volume (MCV) [FPNotebook]. Normal values for MCV are 80-100 fl [MedlinePlus RBC]. Typically iron deficiency anemia has increased RBDW and decreased MCV, whereas folate and vitamin B12 deficiencies have both increased RBDW and MCV.

In addition, blood plasma separation has been demonstrated by Kang et al. who used a microfluidic channel system as a continuous flow blood plasma filter [Kang 2014]. They had 30 μm silicon pillars with 0.7-0.9 μm gaps as can be seen in the schematic Figure 17. Blood plasma is a common sample in healthcare laboratory tests. It is also possible to use acoustic force for blood plasma separation in a microfluidic channel. Lenshof et al. filtered blood plasma with acoustophoresis to get less than 6.0·10^9 red blood cells per litre [Lenshof 2009]. Their set-up also had a distinct prostate specific microarray chip for the separated blood plasma that was able to detect a prostate specific antigen at clinically relevant levels of 0.19-21.8 ng/mL.

![Figure 17. Top view of microfluidic blood plasma pillar filter. Redrawn from [Kang 2014].](image)

Microfluidic cell filtering chips are commercially available. Aquamarijn Micro Filtration offers silicon membrane type microsieves that comprise of silicon chips of 5x5 mm² with 0.6 mm thickness and pore sizes ranging from 200 nm
Innosieve uses the same microsieves, but they have developed their own equipment for Salmonella and Legionella bacteria detection [Innosieve]. The analysis is based on cell filtering and fluorescence labelled cells which are detected with a MuScan light emitting diodes (LED) based fluorescence scanning system. It takes about 200 overlapping scans and makes image analysis for application-specific size differentiation.

1.2.2 Chemical applications

Capillary electrophoresis

Capillary electrophoresis (CE) which allows the separation of molecules in electrical field as a function of size and charge was developed by Virtanen in 1974 [Virtanen 1974]. Traditional CE is performed using glass capillary fibers, which are typically 50-70 cm long and have an inner fiber diameter of 50 μm, although 25 and 75 μm are also used as the capillary length and inner diameter depend on separation method. It is also possible to coat capillary surface with some polymers, surfactants or small molecules before separation. This is done to decrease electro-osmotic flow or sample-capillary wall interaction. In the measurements the capillary fiber is first filled with separation buffer, next the sample is injected either electrokinetically or with pressure from one end of the fiber. The electric field is coupled after injection and separation can begin.

Glass capillary CE is used for many different applications including: DNA, clinical and forensic samples, carbohydrates, inorganic anions and metal ions, pharmaceuticals, enantiomeric species and proteins and peptides [Altria 1999].

There is at least five separation modes in CE [Jorgenson 1986] [Ewing 1989]:

- capillary zone electrophoresis
- isotachophoresis
- capillary electrokinetic chromatography
- capillary gel electrophoresis
- isoelectric focusing

All of these have also been realized using microfluidics.

Microchip CE was first developed at the beginning of 1990’s by Andreas Manz and D. Jed Harrison [Manz 1992] [Harrison 1992]. A few hundred volts/cm is used both in microchip and in capillary fiber systems and the idea behind microchip CE was to get more precise injection than with existing glass capillary fibers. Figure 18 shows a schematic representation of microchip CE injection and separation. First, all channels are filled with a separation buffer. Next the sample is put to reservoir and an electrical field is applied between A and B for sample injection. The sample will fill whole injection channel from A to B, because the channel volume is significantly smaller than volume of reservoir A.
Injection high voltage (HV) is stopped and the injection cross now has nL sample volume (0.125 nL with 50x50 μm² channel and direct injection cross). The electrical field is then coupled between C and D for CE separation. The sample from the injection cross moves towards reservoir D and separated molecules are detected at the end of the channel.

![Sample injection](image1)

**Figure 18.** Schematic microchip CE: AB is typically ~1 cm and CD ~5 cm.

Precise injection makes it possible to use shorter separation channels of a few centimeters, compared to 50-70 cm range glass capillary fibers. With glass capillary fibers you can use sample injection only from the one end of the capillary, whereas with microchannels it is possible to make sample injection channel crosses. Injected samples are much shorter (<0.1x) in the injection cross of a microchip channel than in capillary fibers. Moreover, the short separation channel of microchip means also means a shorter separation time and smaller HV source, for example if the HV source with a glass capillary is 30 kV, it could be low as 3 kV with the equivalent microchip. Separation times in traditional glass capillary CE are tens of minutes depending on the separation method, in contrast separation times with microchip CE are minutes or below. Resolution is usually better with traditional CE, because of long separation time and separation capillary. This is generally outweighed by the benefits of microchip CE like faster analysis and the possibility to integrate sample preparation in the same chip, which makes microchip based CE systems also much smaller than with glass capillary fibers.

Microchip CE has been implemented in numerous different polymers: Effenhauser et al. developed PDMS CE chip [Effenhauser 1997], whilst Graß et al.
developed Polymethyl methacrylate (PMMA) chip for isotachophoresis [Gräß 2001]. Thick resist SU-8 has also been used for CE separation in [Publication V], whereas low cost roll-to-roll (R2R) PMMA chips have also been developed [Publication II].

Microchip CE has been used for many different applications. Wooley et al. developed high-speed DNA sequencing on 3.5 cm glass microchip and got faster sequencing with the microchip than with slab gel or capillary electrophoresis. [Woolley 1995]. We made the separation of β-blockers [Publication VI] on PMMA microchip by connecting isotachophoresis (ITP) preconcentration to capillary zone electrophoresis. Microfabrication also makes it possible to integrate electrochemical detection (ECD) to microchip CE, for example Chen et al. have demonstrated amperometric and conductivity detection modes for microchip CE-ECD in environmental pollutant applications [Chen 2006]. The author of this thesis has also developed testosterone detection on a silicon-glass microchip and also integrated thin film electrodes for HV and conductivity detection [Publication IV]. Vrouwe et al. have utilized a glass microchip with thin film electrodes for HV and conductivity detection [Vrouwe 2004] [Vrouwe 2005]. This system was further modified by connection to a portable reader to allow lithium POC testing at home [Vrouwe 2007] [Floris 2010]. In addition, microfabrication provides the ability to couple DNA amplification and CE. Mathies’ group has developed portable glass microchip PCR-CE for pathogen and infectious disease measurements [Lagally 2004]. Microchips have also allowed the development of a portable CE instrument system for space applications e.g. amino acid biomarker detection on Mars [Skelley 2005].

It is possible to buy microfluidic CE chips and measurement systems from many manufacturers like Micronit [Micronit], Dolomite microfluidics [Dolomite] and Microfluidic ChipShop [Microfluidic-chipshop]. These are more for research use, because some microfluidic instrumentation is needed. Both glass and polymer chips are available. There are also complete systems: Agilent’s Bioanalyzer is one example and it was the first commercial system for microchip CE [Agilent]. It uses glass microfluidic chips for gel-based CE that possess different characteristics for example DNA/ribonucleic acid (RNA), protein, cell applications. The sample size is for these devices is 1-4μL and is currently available for research, but it is not offered for clinical use.

**DNA amplification**

Polymerase chain reaction (PCR) is a technology to amplify DNA from a single copy or a few copies exponentially several orders of magnitude. PCR was invented in 1983 by Kary Mullis and is based on temperature cycling between 50°C and 100°C [Mullis 1986]. PCR typically has three temperature steps in each cycle: denaturation (90-100°C), annealing (50-65°C) and extension (72-80°C). The denaturation step melts DNA into two single-stranded DNA segments and it is in the annealing step that the primers - which define the DNA
sequence for amplification - bind to the single-stranded DNA. Polymerase enzyme copies defined part of DNA in the extension step and this three step cycle is repeated 20-40 times. Today PCR is a common step in molecular biology studies e.g. hereditary diseases, forensic science, DNA paternity testing and infectious diseases. The whole amplification process typically takes about 2 hours in traditional PCR tubes with thermal cyclers. This long reaction comes from the slow heating and cooling rates of the thermal cyclers that are typically only a few degrees per second.

Microchip PCR reaction was first developed on silicon by Northrup et al. [Northrup 1998]. Silicon has well established microfabrication technology and silicon has a good thermal conductivity of around 150 W/(m·K). This good thermal conductivity means fast heating and cooling that is main advantage in contrast to traditional methods. Microchip PCR process time is only a few minutes, because tens of degrees per second heating and cooling are possible.

Polymer chips (for example PDMS, PMMA, SU) have also been utilized for PCR [Zhang 2006]. Even though polymer materials tend to be thermal insulators, e.g. PMMA thermal conductivity is 0.17-0.25 W/(m·K) [Engineering toolbox], it is still possible obtain fast rates of heating/cooling by using thin-walled reaction chambers.

Moreover, it is also possible to couple integrated thin film heaters and a temperature measurement element (thermistor) on silicon or to couple complementary metal oxide semiconductor (CMOS) thin film elements with intelligent temperature control [Erill 2004]. However as these PCR systems should be disposable it precludes the use of these CMOS features as it is cheaper to integrate a thin film heater/thermistor set-up on the silicon and put temperature control outside of the microfluidic device. In contrast, it is more of a challenge to integrate heater and thermistor elements on polymer materials, but that is not necessarily needed. It is possible to integrate these heater and temperature measurement elements into thermal cyclers thus the resulting microfluidic chip is much simpler and cheaper. Good thermal contact is then needed between the microfluidic chip and heater/thermistor elements.

ST microelectronics developed one of the first commercial silicon based PCR chip system ten years ago [Consolandi 2006]. This In-Check prototype is shown in Figure 19 with the silicon part on the right hand side. This device has four 2 μL PCR reaction chambers. This is similar to the GenSpector® TMC-1000 silicon PCR chip system developed by Samsung with a 1 μL chamber [Cho 2006].
DNA amplification is often just one analysis step in a LOC device as sample pre-treatment and mixing can also be combined on the same microchip. Chang et al. used EWOD controlled droplets for sample mixing and PCR was present on the same chip in a separate chamber as part of a successful Dengue II virus detector [Chang 2006]. On the other hand, Min et al. integrated DNA concentration, purification and amplification on the same chip, which featured a pillar packed chip for DNA capture and concentration on pillar surfaces [Min 2011]. DNA of Escherichia coli bacteria was captured with a kosmotropic salt and by hydrogen bonding to a silicon oxide surface. Concentrated and purified DNA was then released with PCR mixture. Sample preparation was done in a centrifuge and amplification in GenSpector® TMC-1000.

Microfluidics has also been utilized with traditional PCR instruments, for example, Hindson et al. used microfluidics for droplet generation and detection [Hindson 2011]. Sample droplets were generated in an oil carrier and remained as separate droplets through whole process. DNA amplification of droplets was carried out with a 96-well plate PCR instrument. In contrast, Shen et al. used SlipChip microfluidics to generate 1280 droplets with 2.6 nL volume [Shen 2012]. SlipChip microfluidics means slipping bottom and cover plates. Both parts had microfluidics channels and chambers and the slipping process made connections between channels and chambers that generated the droplet array. A traditional PCR cycler was subsequently used for the DNA amplification.

Microfluidics can also make the use of continuous flow PCR possible as demonstrated by Kopp et al. who presented the use of constant temperature heaters [Kopp 1998]. The microfluidic channel was arranged such that it past 20 times through three constant temperature zones: 95°, 77° and 60°. A sample size of 10
μL was used for the DNA gyrase gene of Neisseria gonorrhoeae and by varying the flow (5.8 to 72.9 nL/s), analysis time could be between 18.7 to 1.5 min.

Isothermal DNA amplification has also been implemented using microfluidics and it is especially easy for polymer chips as only a constant temperature is needed [Craw 2012] [Chang 2013]. Isothermal amplification is not a quantitative analysis like PCR but it is suitable for POC analysis when a rapid yes/no result is required like whether antibiotics should be administered or not.

More recent application areas for microfluidic PCR has been in virus and bacteria detections. Yamanaka et al. have developed a flow through type PCR chip for the influenza A virus [Yamanaka 2011]. The process involved 30 cycles and 15 min amplification before a 5 μL sample of the resulting amplification was analysed by a separate electrochemical detection chip. They added methylene blue to the sample as an electro-active material for the electrochemical detection, where current is measured with linear potential sweep in squarewave voltammetry. A peak current of the electrochemical detection decreases, when methylene blue binds to amplified RNA. Methylene blue has high affinity to amplified RNA but not to PCR reagents. In contrast, Zhu et al. measured H5 avian influenza virus with a microfluidic PCR chip that featured 20 μL PDMS chambers for amplification, real-time fluorescence detection and a total analysis time of 65 min. [Zhu 2014]. Moreover Jiang et al. have presented a continuous-flow PCR method for bacteria analysis [Jiang 2014]. In their work they used five different bacteria: Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Enterococcus faecalis. Their PDMS chip featured 33 cycles and amplification took a total of 25 min.

Commercial chips for DNA amplification are available: Veredus Laboratories for instance, has now commercialized ST Microelectronics’ In-Check system - under the name VereChip technology - and it has applications for flu epidemics, tuberculosis, tropical diseases, pathogens and foodborne diseases [Veredus]. Also RainDance offers the quantitative droplet based RainDrop digital PCR system which can handle almost five million samples per minute in eight channels. [RainDance].

1.2.3 Biological applications

Cell culturing

In vitro cell culturing means that the cell culturing is done outside a living organism, typically in a Petri dish. This method makes cell studies simpler and faster, whilst also reducing the need for human and animal trials. The drawback is that the in vitro culturing environment does not always correspond to real environment. Living organism are much complex systems, where many cells, proteins, genes and organic compounds interact with each other. Cell behavior in vitro system might be totally different than in real environment because of mechanical, thermal, chemical and flow conditions. The use of microfluidic
Microfluidics structures however, allows fluid or gas flows and environmental or stress conditions to be mimicked [Berthier 2012]. In addition, microfluidics is a versatile enabling technology for cell based research as it makes high throughput screening (HTS) with cell microarrays possible [Mu 2013]. Also even though Petri dishes are already available in multi-well formats, microfluidic structures can provide much denser cell arrays of a few thousand measurement spots that is at least 10x more than is possible with multi-well plates. Surface patterning is another way to carry out cell microarrays and hydrophilic patterned spots with microfluidic cell medium channels have been used for cell culturing [Kolari 2009a].

Cell microarray size is not the only beneficial feature of microfluidics. More critical are the fluidic features [Young 2010] and we have made microfluidic channels for cell feeding and culturing [Kolari 2009a]. Such feeding channels for cell medium make long-term measurements practical as although it is possible to add cell culturing medium to Petri dish, with microfluidics it can be automated. Cells also behave differently in a flow system than in static Petri dish and a medium flow system is also closer to the real environment of the human body.

Stress conditions can also be induced by using microfluidic fluid flow based gradients, diffusion or just fluid pressure. Tanake et al. studied shear stress effects on human hepatocyte cells by using 100-200 μm² quartz glass channel made with a CO₂ laser. [Tanaka 2006]. A single layer of hepatocyte cells were cultured on surface of the microchannel and the cells were subject to shear stress induced with flow velocity or with viscosity of the perfusion solution because laminar flow has zero velocity on channel surface. Two types of experiments were performed: the first had a constant flow rate 2.5 μL/min and viscosity was changed between 1-12 mPa·s, which means 0.14-1.6 Pa shear stress. Viscosity was tuned with dextran. Non-toxicity of dextran was tested before shear stress experiment. Cells were normal after one week culturing with dextran-added medium. The second experiment had a constant viscosity value 12 mPa·s, whilst flow rate was changed to between 1-10 μL/min – equal to 0.6-6 Pa shear stress. It was found that cells kept their static shape under 0.5 Pa shear stress and stretched in the flow direction with 0.6-1.2 Pa shear stress. Above >2 Pa shear stress cells were round shaped and flowed away after approximately one day.

Surface strains and fluid flow stresses were also measured by Sinha et al. [Sinha 2015]. In their research they developed a PDMS chip with air pressure actuator induced surface strain. They used C2C12 mouse myoblast cells on an 80 μm thick PDMS membrane and measured combinations of five strains and five shear stresses between 2-20% equibiaxial strains and 0.18-0.33 Pa shear stresses. It was possible to measure all these combinations at the same time with four replicates as the PDMS membrane had in total 100 circular measurement units with 1.5 mm diameter.
A microfluidic cell culturing system with medium flow channels has been made on a PDMS with SU-8 mould by Hung et al. (Figure 20a) [Hung 2004]. This system also had separate channels to produce a concentration gradient of the reagents (Figure 20b). The concentration gradient was demonstrated with coloured dye liquids. Red dye was first used as cell culturing medium in the measurements. It brings fresh medium to every chamber, whereas yellow and blue dye gradients were used as the cell suspension and reagents in the measurements, respectively. Yellow and blue gradients show that lateral flow between columns was minimal and that a linear reagent gradient can be generated. Human carcinoma cells were cultured with continuous medium flow at 37°C in an incubator. Calcein AM – which gives a fluorescent signal only in living cells after activation by intracellular enzymes - was then introduced as a reagent to demonstrate the cell-based assay. It was seen to activate in 10 min and gradually disappears over 10 days following removal of calcein AM. The fastest measured cell doubling time was ~1.4 days with 0.12 μL/min medium flow and in the beginning chambers had 20-40 cells. After ~10 days chambers were full of cells and passing of cells that makes long-term measurement possible was demonstrated with trypsin flow. A reduction of cell number was observed in 24 hours and chambers were once again ready for a new cell culturing cycle.

**Figure 20.** Microfluidic cell culturing system with 10x10 chambers [Hung 2004]. 

(a) Perfusion inlet and outlet are for medium flow. Two inlets in the left are for cell feeding and concentration gradients of reagents. 

(b) Concentration gradient was demonstrated. First the red dye is pumped left to right. Then yellow and blue dyes are pumped up to down with concentration gradient generator.

There are also commercial products for cell culturing: Fluidigm has integrated fluidic circuits (IFC) for single cell analysis [Fluidigm], whereas Cytoo make micropatterned CYTOO chips and CYTOO plates that direct cell culturing [Cytoo].

**Electrical cell microchips**

In electrophysiology cells are stimulated as well as measured electrically. It is possible to electrically open voltage-gated the ion channels of cell membrane. For example, Fromherz has made ion channel measurements with nerve cells and developed also two types of devices: capacitors to stimulate ion channels and transistors to measure ion channels. [Fromherz 2008]. Microfabrication
made it possible to make these devices on the same scale or even smaller than nerve cells ~10 μm. These devices are also available in an array format with as a small spacing.

Electroporation with electric field has been used to increase permeability of cell membrane and was first demonstrated with mouse cells in 1982 by Neumann et al. [Neumann 1982]. In their experiments they used an 8 kV/cm electric field and 5 μs pulses to inject herpes simplex thymidine kinase gene into mouse cells. The first micro-electroporation was demonstrated by Huang and Rubinsky [Huang 1999]. They developed transparent 1 μm thick silicon nitride (SiN) membrane with one 2-10 μm hole for cells. This SiN membrane was between two fluidic chambers with electrical contacts. Micro-electroporation system was tested with human prostate adenocarcinoma cells and rat hepatocytes. Pulse length and amplitudes were 2 μs to 100 ms and 0-120 V, respectively. Transition from plugged mode to electroporation mode was observed between 20-35 V with rat hepatocytes and 60 ms pulses at 4 μm hole. Microfluidic electroporation makes it possible to take intracellular samples like nucleic acids, proteins and metabolites to analysis; or to inject genes, drugs and nanoparticles inside cells [Geng 2013]. Lu et al. have also used microfluidic electroporation for cell lysis to subcellular analysis [Lu 2004]. Microfluidic electroporation has also several advantages to batch type electroporation in a cuvette. It is possible to control each individual cell and several cells parallel. A lower electrical field is needed with the microfluidic system, because the distance between electrodes is smaller. Batch type electroporation is simpler for industrial processes.

Sonoporation has been also used to increase the permeability of the cell membrane. Longsine-Parker et al. combined piezoelectric transducer driven sono-poration and electroporation in the same microfluidic channel [Longsine-Parker 2013]. They had three-dimensional structure where both sonoporation and electroporation was in an orthogonal direction, which they combined with a propidium iodide/Calcein AM solution in order to test cell poration with human cervical cancer (HeLa) cells. The results of this simultaneous sonoporation and electroporation showed a 95.6% average poration efficiency and 97.3% cell viability. Electrically actuated sonoporation is not the only option for cell poration as demonstrated by Le Gac et al. who used optical laser actuated sonoporation and which makes the construction of the microfluidic chip much simpler [LeGac 2007]. They used laser to generate cavitation bubble, which induced cell membrane poration. They defined distance 0.75x bubble radius that produced >75% poration probability. In their study they measured Calcein AM release/trypotan blue uptake from HL60 (human promyelocytic leukemia) cells and achieved >75% poration probability with 532 nm Neodium:YAG laser. Laser pulses were 6 ns long and laser energy at focal point was 10-100 μJ, which generated bubble lifetimes of 10-30 μs.

Patch clamp is a technique to measure molecular transport through the ion channels of a cell membrane. Glass capillary based patch clamp was developed
in the late 1970s. The advent of microfabrication now makes it possible to perform high throughput patch clamp with multiple parallel channels and both vertical and horizontal patch clamp have been demonstrated with microfabrication. Lehnert et al. used silicon dioxide (SiO2) nozzles down to 2.5 μm diameter on silicon wafer for patch clamp and characterized these nozzles using Chinese hamster ovary (CHO) cells [Lehnert 2002]. Ionescu-Zanetti et al. on the other hand fabricated lateral PDMS capillaries for patch clamp [Ionescu-Zanetti 2005]. Their device had 12 PDMS capillaries in 2x6 array format, where one cell had one 3x4 μm² channel and they also used mammalian CHO cells for potassium K+ channel measurements. In contrast, Yobas et al. developed lateral glass capillary array for patch clamp [Ong 2006] [Ong 2007] and which was subsequently integrated in a standard 1536-well microplate format [Tang 2010]. One cell had 12 glass capillaries in 2x6 array and four fluidic connections. Each 1536 microplate had 96 units for patch clamp measurements. Each glass capillary had 1.5 μm inner diameter and mammalian rat PC12 and rat basophilic leukemia (RBL) cell lines were used to potassium channel studies.

In this work the author has been involved in the development of electrical stimulation for hERG-CHO-K1 hamster cells [Publication III], heart stem cells [Publication III] and SH-SY5Y human neurological cells (neuroblast from neural tissue) [Kolari 2010a]. Our stimulation system is shown in Figure 21a and features titanium electrodes are on bottom of 96-well plate. These electrodes have a 5-10 nm thick TiO2 layer which is self-oxidised in atmospheric conditions. In Figure 21b, neurological cells are present on the titanium electrodes and a stimulation amplitude utilized was between 1-20 V. In publication III heart stem cells were used with finger type Ti-TiO2 electrodes and a 4 V stimulation amplitude. Moreover, it was possible to change heart beat rate with electrical stimulation [Publication III].

![Figure 21. a) Measurement interface in the 96-well plate format. b) SH-SY5Y human neurological cells on titanium electrodes. a) From publication [Kolari 2010a] and b) related to [Kolari 2010a].](image)

Microelectrode arrays (MEA) are offered by several companies like Axion Biosystems [Axion] and Multi Channel Systems MCS GmbH [Multi Channel]. These MEA supports are used for electrophysiological measurements of cells. Cellectricon also provides the Cellaxess Elektra system as well as cell-based screening services to accelerate drug discovery [Cellectricon].
1.3 Fluorescence detection for microfluidics applications

Fluorescence detection has been one of the most common detection technology for microfluidics [Kuswandi 2007]. It has better detection sensitivity than other optical detection technologies for example absorption, bioluminescence or chemiluminescence. Fluorescence detection is also easier to couple to microfluidic device than electrical measurements like conductivity or electrochemical detection, because measurement electrodes are not needed.

Fluorescence phenomenon was found already in 1560 and 1565 by Sahagún and Monardes, respectively [Azuña 2009]. Sahagún reported that Aztec healers had seen blue colour at a plant coatli, which was used to treat urinary disorders and Monardes found blue fluorescence from the Mexican medical wood Lignum nephriticum.

Figure 22 displays the nature of fluorescence and phosphorescence as a Perrin-Jablovski diagram - first outlined by Perrin in 1929 and Jablowski in 1933 [Jablonski 1933] [Valeur 2011]. Electrons are excited to the singlet $S_1$ state by absorption of light in a process is called fluorescence excitation. This absorbed energy is higher than the excitation state energy and part of electrons return back to $S_0$ state by non-radiative decay and part with fluorescence emission. The wavelength of the fluorescence light is longer than the wavelength of the excitation light, because excited electrons pass vibrational relaxation before fluorescence emission. The timescale of fluorescence is typically from ns to ms, for example the lifetime of europium dye is about 1 ms.

![Figure 22. Fluorescence and phosphorescence is shown in Perrin-Jablonski energy-level diagram. Fluorescence excitation must have higher energy than fluorescence and phosphorescence emissions that excitation can happen. Fluorescence and phosphorescence have always lower energy than excitation light, because excited electrons pass vibrational relaxations and intersystem crossing before emission.](image-url)
A part of $S_1$ electrons undergo intersystem crossing to the triplet $T_1$ state. This intersystem crossing is a non-radiative process, where an electron’s spin is reversed resulting in the triplet $T_1$ state which has the same or a little bit lower energy than the corresponding singlet $S_1$ state.

Phosphorescence appears when the triplet $T_1$ electrons decay by phosphorescence to the singlet $S_0$ state. Again, some $T_1$ electrons have non-radiative decay to $S_0$ state. Phosphorescence typically has a longer lifetime than fluorescence varying from hundreds of nanoseconds (zinc sulfide) to several hours.

Laser induced fluorescence (LIF) means that laser is used for fluorescence excitation. A typical LIF measurement setup developed by author is shown in Figure 23 [Orellana 2001]. It has laser excitation and photomultiplier tube (PMT) detection (Figure 23a). The laser excitation is at a $\sim 45^\circ$ angle to avoid laser scattering to the PMT (Figure 23b) and the fluorescence light is collected perpendicularly upward before being filtered in the front of the PMT.

**Figure 23.** a) Schematic LIF measurement setup. b) Photo of LIF measurement setup. From [Orellana 2001].

The difference between the excitation and emission wavelength is known as the Stokes shift [Lakowicz 2006] and usually in fluorescence measurements the Stokes shift is just a few tens of nanometers. Typical band pass filters have 10-20 nm bandwidth and thus crosstalk from excitation to emission is possible.
Fluorescence detection has a number of variations, one of which is Förster resonance energy transfer (FRET) where a sample has two fluorophores that are within 10 nm of each other [Joo 2008]. The first fluorophore is excited and the fluorescence energy is transferred to a second fluorophore via nonradiative coupling before the fluorescence emission of the second fluorophore is finally detected. Typically FRET makes Stokes shift larger and fluorescence light filtering easier and we have used FRET for CRP measurements with europium and CY5 fluorescence labels [Orellana 2001]. Europium was excited with a helium-cadmium laser at 354 nm and detected with a photomultiplier tube at 670 nm. This meant a 316 nm Stokes shift, which is an excellent value for fluorescence filtering as it offers high measurement efficiency. Gérard Mathis used also europium, but coupled to allophycocyanin (APC) as the second fluorophore [Mathis 1995]. He excited europium at 337 nm and measured the fluorescence of APC at 665 nm with a 328 nm Stokes shift in order to probe molecular interactions. Moreover, Ha et al. have demonstrated single molecule efficiency with near-field scanning optical microscopy (NSOM), which makes a nanometer scale imaging possible [Ha 1996]. This was achieved by combining tetramethylrhodamine and Texas Red fluorophores. Tetramethylrhodamine was excited at 514 nm by an argon laser and the fluorescence of Texas Red was measured at 613 nm with an avalanche photodiode (APD). This tetramethylrhodamine-Texas Red FRET was used to probe the interaction between two single molecules.

A second variation is time-resolved fluorescence: if a fluorophore’s emission lifetime is long >50 μs, it is possible to measure fluorescence after excitation light pulse [Orellana 2001]. It is also possible to combine FRET and time-resolved fluorescence as shown by the author built this time-resolved microarray reader for FRET measurement (Figure 23a). Our setup had a 50 μs delay from the excitation pulse and a 400 μs measurement window (Figure 24a). This system was used for CRP measurements with europium-Cy5 fluorescence labels and as europium has a long 1 ms lifetime it made these time-resolved measurement possible (Figure 24b). In addition, our system was able to be based on mechanical choppers, because of this 1 ms fluorescence lifetime. The use of fast pulsation of diode lasers and fast detectors makes a totally electronic based time-resolved measurement possible with shorter fluorescence lifetimes. This has been demonstrated by Waddell et al. who used a <1 ns fluorescence lifetime measurement with a 780 nm pulsed diode laser and APD detector [Waddell 2000]. The timing response of the device was 275 ps. Also the use of fast time-resolved detection made the fluorescence lifetime measurement possible and they were able to measure a fluorescence lifetime of 1.21 ns for the tricarbocyanine fluorophore.
The third variant is evanescent field excitation where excitation light is coupled into an optical waveguide on the sample surface. The evanescent field of the excitation light penetrates few hundred nm from the surface and excites fluorophores that are attached to the sample surface (Figure 25a). Excitation light is coupled to the optical waveguide, but fluorescence emission goes to the whole solid angle which makes it possible to measure just fluorescence emission almost without noise from the excitation light. Excitation light coupling is the main challenge for evanescent field detection and side and grating couplings are typically used. Grating coupling is presented schematically in Figure 25b. We have developed grating coupled evanescent field excitation with silicon nitride (SiN) waveguides and have measured the linear fluorescence response with fluorescein label concentrations from $10^{-3}$ to $10^{-9}$ M [Kolari 2006a]. In contrast, Pawlak et al. used evanescent wave excitation for microarray detection [Pawlak 2002]. They dispensed 400 pL droplets with 140 μm diameter on an 150 nm
tantalum pentoxide (Ta$_2$O$_5$) waveguide coated glass waveguide and excitation light penetrated about 200 nm up from the surface. The detection limit was tested with Cy5-labelled antibody and found to 20 pM with a dynamic range of six decades. To compare, the detection limit detailed by Pawlak et al. was better than our, but the dynamic range was the same. Overall, both the waveguide materials - SiN/Ta$_2$O$_5$ - and the laser excitation wavelengths were different (our 488 nm, Pawlak 492/532/635 nm), though both equipment used Cooled Charge Coupled Device (CCD) camera detection. It is also possible to use polymer waveguides for evanescent wave excitation as demonstrated by Xu et al. who developed a PMMA waveguide on microfluidic channel [Xu 2007]. They dispensed 330 pL droplets with a 150 μm diameter on optical waveguide and measured Alexa Fluor 660 labelled BRCA1 gene (a biomarker for breast cancer) with a 1 μM detection limit. To compare with our own measurements, the waveguide material was different SiN/PMMA and laser excitation was with different wavelengths (Our 488 nm/Xu 675 nm), though once again both works used CCD camera detection.

The final example of fluorescence techniques is two-photon excitation, which combines two photons and excites one photon. Two excitation photons have typically the same energy and excited one photon have the energy that is a sum of those two excitation photons. Excited emission photon have an effective wavelength that is half of those two excitation photons. Most compounds absorb at 210 nm wavelength, which makes label-free native fluorescence measurement possible. For example, Schulze et al. used a 420 nm titanium sapphire laser for
210 nm excitation to measure small aromatics and proteins with borofloat and fused silica glass CE chips [Schulze 2007]. Small aromatics serotonin, 3-phenoxo-1,2-propandiol and tryptophan have native fluorescence emission above 300 nm whereas borofloat glass is intransparent below 300 nm and transparent above 300 nm. Fluorescence excitation of small aromatics was possible however with borofloat chips because the two-photon excitation at 210 nm happened just inside the CE channel in 40 μm focal point. Schulze et al. also measured a protein mixture with lysozyme, trypsinogen and chymotrysinogen, which have native fluorescence in the 270-370 nm wavelength range. In this case, more expensive fused silica chips were used as they are also transparent in the ultra violet (UV) wavelength area (200-400 nm).

LIF is the most common fluorescence technique in microfluidics because lasers offer enough excitation power in small microchannels. LIF detection is an especially good method for microchip CE, because typically 1-100 mW excitation light intensity is needed to produce a 100 μm spot in a 50-100 μm separation channel. It is not possible to get a sufficient excitation light intensity from a lamp or LED, but it is relatively easy with a laser. PMT is typically used as LIF detector, because it offers the best sensitivity, although CCD and Electron Multiplying Charge Coupled Device (EMCCD) are also used as LIF detectors even though they have inferior measurement efficiency but imaging has other benefits. In addition, it is possible to measure several measurements spots at the same time and follow liquid flows or air bubbles in a microfluidic device. Light emitting diodes (LED) can also be used in applications where the excitation area is bigger and LED intensity is enough, but laser excitation is still needed with small <100 μm microchannels.

Fluorescence is widely utilized in many microfluidic applications, for example DNA amplification, CE and cell measurements. Real-time fluorescence DNA amplification monitoring was developed in the 1990’s [Higuchi 1993] [Heid 1996]. SYBR Green gives a fluorescence signal only when it is bound to a double-stranded DNA and in this way it is possible to monitor the amount of DNA in real time. As a result amplification product measurement is not necessarily needed after thermal cycling and amplification chambers could remain closed thus reducing the risk for DNA product contamination.

It is also possible to use electrical measurement to monitor the amount of DNA present, for example with an electrochemical sensor [Patterson 2013]. In reality, fluorescence detection is much simpler for microchip fabrication, because you do not need to integrate any electrodes on the chip as is the case with an electrochemical sensor. Fluorescence detection makes the measurement equipment more complicated and also a fluorescence dye is needed to monitor the amount of amplified DNA, but an electroactive intercalating molecule is also needed for the electrochemical sensor to monitor DNA amount.
A four colour LIF detector was developed by the Mathies’ group for both glass capillary [Kheterpal 1996] [Scherer 1999] and microchip CE [Shi 1999]. This rotating fluorescence LIF-detector for microchips is presented in Figure 26. The LIF-detector had a 70 mW argon laser with a 488 nm wavelength coupled to a four colour PMT and was used for monitoring DNA sequencing in a microplate, which had 96 radial CE channels. Each of the four nucleotides adenine (A), guanine (G), cytosine (C) and thymine (T) had its own fluorescence label. Fluorescence emission wavelengths for each nucleotides were C: 520 nm, T: 550 nm, G: 580 nm and A: >600 nm. This four colour system increased the throughput of the sequencing system, because all labelled nucleotides were measured at the same time and in the same separation channel.

Figure 26. Confocal four colour LIF-detector for glass based CE chips [Shi 1999].

Fluorescence based sequencing chips are much simpler than new microelectronics based sequencing chips, because they do not need any detection electrodes on chip. New microelectronics based sequencing chips are called next generation sequencing microchips. Next generation sequencing systems are however smaller and faster, which will make total sequencing costs lower. There are already some commercial next generation sequencing products, for example
Microfluidics has a digital microfluidics based NeoPrep system and it prepares libraries for next-generation sequencing platforms [Illumina]. Life technologies has Ion Torrent technology which is a semiconductor chip based system which measures pH values to detect nucleotides [Life Technologies]. In addition, Oxford Nanopore Technologies has a nanopore based usb-device for gene sequencing that measures current from nanopore while DNA strand is in transit thru the pore and identifies nucleotides [Nanopore].

Microfluidic CE also has conductivity and electrochemical detectors, though as detection is application specific and it is not possible to label every sample with fluorescence label nor do all samples do have electrical response. Liu et al. used conductivity detection for heavy metal ion measurements using PMMA chips with contactless titanium and gold electrodes [Liu 2012]. Dossi et al. used electrochemical detection for biogenic amines and their amino acid precursors [Dossi 2011]. They integrated electrochemical detector featuring a platinum counter electrode, silver reference electrode and ruthenium-modified glassy carbon working electrode to the end of a commercial glass chip.

A jellyfish green fluorescent protein was used for studying ion channel expression by Marshall et al. [Marshall 1995]. They opened ion channel with electrical stimulation and cells were transfected with the green fluorescent protein. Ion channel measurements have also been performed electrically by exploiting on chip transistor integration [Fromherz 2008]. Overall, fluorescence makes electrical measurement system simpler, because you only need to integrate stimulation electrodes for ion channel measurements. In this work, voltage sensitive fluorescence label has also been used (FLIPR membrane potential assay, Molecular Devices Inc.) for potassium ion channel measurements of hERG-CHO-K1 hamster cells (CYTOMYX, Cambridge, UK, cat: CYL3002) [Publication III]. CHO cells were stimulated with an electrical voltage between 0 and 7 V and the resulting fluorescence was detected. It was found that the fluorescence signal increased 59% and eventually saturated above the 7 V amplitude showing that full depolarization of membrane potential was achieved. About 30 pulses with a 500-1000 Hz frequency was determined to be enough for the stable voltage-dependent fluorescent signal. A lower 4 V amplitude was selected for human embryonic stem cells (hESC) and the results of these experiments showed that it was also the maximum value for long-term stimulation.
2. Microfabrication

Microfabrication technologies for microfluidics have been intensively developed over the last 20 years. For example, microchannels, cavities and holes are the basics structures for microfluidics and silicon and glass were the first materials to be used as they had been previously developed for semiconductor and MEMS applications. Polymer materials came later especially for disposable healthcare applications, but silicon and glass technologies are still used widely in many areas including medical uses.

2.1 Silicon microfluidics

Throughout this thesis deep reactive ion etching (DRIE) is used for silicon microfluidics fabrication [Kolari 2005] and although wet etching is also possible, the silicon crystal structure limits geometries. In addition, holes, pillars and rounded structures are all possible with DRIE, but not with wet etching methods. Silicon is chemically inert, but it possible to modify surface properties by oxidation, for example. We utilized DRIE to make silicon microneedles with 150 μm and 250 μm diameters [Publication I] (Figure 27). Silicon microneedles were oxidized, because oxide is an inert surface for lipid extraction. Moreover, silicon also has a good thermal conductivity of 150 W/(m·K), if heating or cooling is needed, a fact that was exploited for the silicon PCR chips detailed in [Hokkanen 2003].

![Figure 27. Blunt 150 μm silicon microneedle on 2x2 mm² chip. Related to [Publication I].](image)

The oxidation of silicon was used for many purposes like etch masking, electrical insulation, hydrophilic coating and to prevent PCR inhibition. There are also many ways to produce oxides and chemical, physical, deposited and thermal silicon dioxide were all used. A chemical H₂O₂ and physical O₂ plasma oxidations
both produce silicon dioxide (SiO$_2$) surface of about 5 nm. We also deposited two different oxides on silicon between 0.7 and 3 μm thickness by plasma enhanced chemical vapor deposition (PECVD) and chemical vapor deposition - tetraethyloxysilicate (CVD-TEOS), respectively. The thickness of the thermal oxide was typically about 1-2 μm.

We used thermal oxide, CVD-TEOS and silicon nitride (Si$_3$N$_4$) masks for silicon DRIE. These masks were patterned with reactive ion etching (RIE), after which DRIE was performed and finally the etching mask was removed with a chemical etching step. Bulk silicon DRIE etching was used for example, for microneedles, pillar filters, separation channels and medium feeding channels for cell culturing and chambers for DNA amplification. Microchip CE was developed in [Publication IV], whilst cell chips were investigated in [Kolari 2009a] and the use of a PCR chip was demonstrated in [Hokkanen 2003].

Thermal oxide SiO$_2$ was used for electrical insulation in the CE application [Publication IV]. The thickness of thermal oxide was 2 μm, which allowed a 600 V/cm electrical field in the CE separation. Chan et al. also developed a silicon-glass CE chip that featured a separation channel in the glass part and has thin film HV electrodes in the silicon [Chan 2003]. The silicon part also had SiO$_2$ insulation like our CE chip and they measured a 600 V breakdown value for a 1 μm thick SiO$_2$. They used 25 V/cm electrical field for YOYO-1 labelled Lambda DNA/EcoR I digest marker separation. Typical breakdown value for high-quality SiO$_2$ is 1 kV/μm on planar surfaces [Franssila 2010b].

Thermal and PECVD oxides were used to prevent PCR inhibition of a silicon surface, because non-oxidized silicon surface adsorbs PCR reagents [Kolari 2008a]. Combinations of chemical/physical and deposited/thermal were also tested along with the separate testing of native silicon, fused silica, Pyrex glass and PDMS. Results showed that oxidized surfaces are needed, but polymerase also has some adsorption to oxidized surfaces, which results in a small delay for amplification.

Oxidation was also used for hydrophilic coatings on silicon as the presence of a hydrophilic coating enhances capillary force in small <400 x 400 μm$^2$ microfluidic channels and makes passive liquid pumping possible [Kolari 2005]. Oxidation of silicon was also used for both pillar and membrane filters to enhance a liquid flow through the filter structure.

Plasma deposited Teflon-like fluorocarbon was used as a hydrophobic coating on silicon [Kolari 2006b]. This hydrophobic coating makes it possible to control liquid flows in microfluidic channels, for example to stop liquid flow and act as a passive valve. It is also possible to pattern porous hydrophilic pillar surfaces with this hydrophobic coating on silicon and glass [Kolari 2009b]. First the whole surface is patterned with hydrophilic pillars then the hydrophobic coating is deposited and patterned. This patterning creates hydrophilic microfluidic
channels between hydrophobic areas meaning that liquid moves via capillary force in these patterned hydrophilic pillar channels.

SOI wafers were needed for membrane filters and a HEPA filter on SOI wafer was developed [Kolari 2010b] (Figure 28a) and a thin 10 μm thick SOI device layer operated as filter membrane (Figure 16). Double side lithography and etching were used to create hole sizes in silicon membrane between 700-900 nm after silicon DRIE (Figure 28b) and this hole size was subsequently reduced by thermal oxidation to 350 nm.

![Figure 28. a) Silicon filter for on-line bioprocess monitoring. b) Filter membrane before oxidation with 0.75 μm holes and 2.5 μm spacing [Kolari 2010b].](image)

Aspect ratios of 10:1 (height:width) is typically easy for DRIE, but above this it becomes more and more difficult [Franssila 2010c]. Silicon grass is also a problem in very deep structures that are several hundred micrometers. Silicon grass consist <1 μm diameter and <10 μm pillars in random order. Silicon grass was unwanted side effect for our microfluidic structures, because this porous grass surface increases sample contamination on surface and it is difficult to clean. In our experiments more than 300 μm deep structures were problematic, if silicon grass is not allowed in the bottom of microfluidic structures. High optical absorption of silicon grass is a benefit in optical applications and for that reason it is called black silicon. Electrochemical etching is another way to produce black silicon, which is used e.g. on solar cells, photo detectors and image sensors.

Thin film comprising of molybdenum and amorphous silicon electrodes were tested for heating and temperature measurement [Hokkanen 2003]. A 50 nm thick molybdenum layer was deposited on the surface by sputtering, whereas low-temperature chemical vapor deposition (LPCVD) was used to deposit the amorphous silicon thin film electrodes on silicon wafer (Figure 29). The amorphous silicon was doped by boron ion implantation and annealed at 950°C to activate dopants. Both the molybdenum and amorphous silicon were patterned with reactive ion etching (RIE) to produce stripes that were either 2 μm (molybdenum) or 10 μm wide (amorphous silicon). A heater resistance of 500 Ω and a thermistor resistance of 2000 Ω was determined for both electrode materials.
A glass lid is typically needed for silicon microfluidic channels. Glass has a good optical transmission and a low fluorescence background. We have used both low temperature bonding and adhesive bonding for glass lids [Publication IV]. Low temperature bonding is a wafer scale process and is a very good choice for commercial microchips as the low temperature conditions used (200°C for 2 hours) ensures that any thin film electrodes remain undamaged. Adhesive bonding is more suitable in the research and development phase because it can be used for single chips. In this case, Araldite Rapid (Bostik, Tampere, Finland) screen printing epoxy was used and required a curing time of about 1 hour at 20°C.

The polymer microfluidic interface is one potential fluidic interface for small silicon devices and this was tested by glue bonding a silicon microneedle to a polymer microfluidic interface [Publication I]. The microneedle device comprised of a 2x2 mm² silicon microneedle and 35x42 mm² PMMA chip, which were glue bonded together at 150°C for 1 hour with an epoxy (Epotek 353-ND).

2.2 Glass microfluidics

In our laboratory glass microfluidics have been developed by plasma etching of Pyrex and fused silica [Kolari 2008b]. There is also some other glass materials that are common like borofloat, but they were not compatible to our clean room processes, because they contain e.g. alkali metals. Glass material is very good substrate for microfluidics because it is transparent, an electrical insulator and chemically inert. In addition, wet etching is also possible with glass materials although vertical walls are not possible – unlike plasma etching - because wet etching is isotropic thus, for example, pillar structures are impossible. Etching depth is still a challenge for glass plasma etching as a few tens of micrometers are easy, and few hundreds of micrometers etching has been demonstrated. However, through holes for 500-700 μm thick glass wafers are still under development [Kolari 2008c] and also high aspect ratio (>10:1) pillars are difficult to obtain by glass etching. Sukas et al. have however demonstrated fused silica pillars for capillary electrochromatography [Sukas 2012]. They fabricated 10 μm high foil, diamond and hexagon pillars with ~2 μm minimum spacing. Pillar cross-sections were width 7.5-8.1 μm and length 39-59 μm.
Circular patterns of plasma deposited Teflon-like coatings were placed on a hydrophilic glass surface in order to render them hydrophobic. Prostate cancer cells were then cultured on the exposed hydrophilic glass surface and the Teflon coating acted as a wall between circles [Kolari 2009a] (Figure 30). Cells did not adhere to the Teflon coating and they stayed only on the hydrophilic areas.

![Figure 30. Prostate cancer cells (light areas) surrounded by hydrophobic Teflon-like barriers [Kolari 2009a].](image)

Amorphous silicon thin film electrodes for high voltage supply and conductivity measurements were fabricated on fused silica wafers [Publication IV]. In Figure 31, 10 μm wide conductivity detection electrodes in a 75 μm CE channel are shown. These amorphous silicon electrodes were covered with low temperature oxide (LTO) and the LTO was opened (oblong) around electrodes. In addition, 40 μm wide titanium electrodes for cell stimulation experiments have been developed in this work [Publication III]. Titanium was deposited on fused silica wafer and oxidised at room atmosphere with 5-10 nm titanium dioxide (TiO$_2$). Microelectrode arrays (MEA) are typically fabricated with titanium nitride (TiN), platinum or gold electrodes and not with titanium-TiO$_2$ electrodes as the insulating TiO$_2$ layer is not wanted as it reduces electrical contact. The titanium electrodes detailed above were used for stimulation and it was found that the presence of insulating TiO$_2$ was beneficial. The presence of the dielectric TiO$_2$ layer made capacitive coupling possible between the titanium electrodes and cells which prevented electrolysis and allowed higher stimulation amplitudes to be used. It is also possible to use these titanium electrodes for electrical measurements as demonstrated by Ryynänen et al. who made field potential measurements with titanium electrodes for neuronal cells and cardiomyocytes [Ryynänen 2011]. They compared titanium-TiO$_2$ electrodes to TiN electrodes and found that although Titanium-TiO$_2$ has a higher noise level, performance in field potential measurements of neurons and cardiomyocytes was acceptable.
Figure 31. Amorphous silicon electrodes for conductivity measurement from [Publication IV].

### 2.3 Polymer microfluidics

A SU-8 thick resist was used to fabricate CE chips in [Publication V]. The microfluidic structures are identical to those in the silicon CE chip [Publication IV] but the SU-8 fabrication process is much simpler as the time consuming and expensive DRIE process is not needed as SU-8 only requires a thick resist lithography. The drawback is that a SU-8 resist has a higher fluorescence background than glass, which decreases signal noise ratio 10 to 100-fold [Publication IV].

PMMA was one of the first polymer materials to be used in microfluidics due to its transparency and reasonably low background fluorescence [Abgrall 2007]. Both injection moulding and hot embossing are used for fabrication of microfluidic devices and for example, we used hot embossed PMMA CE chips for [Publication VI].

PMMA has been also used in roll-to-roll (R2R) hot embossing (Figure 32) and we have developed PMMA R2R chips for microfluidic CE [Publication II]. R2R PMMA makes low cost healthcare CE devices possible. Two 125 μm thick PMMA foils were used: a CE channel was hot embossed into first foil and 1.3 mm fluidic access holes were drilled into the second foil. Finally, these foils were laminated by solvent bonding in a separate process step. A 125 μm thick PMMA foil is also good for optical detection as it allows the use of 60x standard microscope objectives as their typical working distance is around 200 μm. In contrast, this short working distance is challenging for glass wafers, because <200 μm thick glass wafers are difficult to handle.
Figure 32. Schematic process flow for R2R hot embossing from [Publication II]. Polymer foil is unrolled from an unwinder unit (a) and fed between a microstructured embossing cylinder and counter pressure cylinder (b). The hot embossed foil is rolled with a winder unit (c). The guide roller (d) and foil speed are used to control pre-heating time before hot embossing.

Hot embossed PMMA polymer was used for a microneedle device, but R2R PMMA can also be used to fabricate disposable devices [Publication I] (Figure 33). Fluidic channels (100 μm deep and 400 μm wide) were hot embossed to a 375 μm thick foil and two 200 μm wide access holes were punched for the microneedle. The microfluidic channels were then lidded with a 375 μm thick PMMA foil, which contains two drilled 5 mm wide holes for pneumatic actuation. Small 200 μm holes and large 5 mm holes were in the opposite side of PMMA chip. Finally the pneumatic actuation holes were covered with a 125 μm thick thermoplastic polyurethane (TPU) membrane.

Figure 33. PMMA polymer microfluidic part for silicon microneedle from [Publication I].
3. **Microfluidic devices and fluorescence measurement systems**

This chapter presents the author’s microfluidic devices and fluorescence measurement systems for microfluidics that have been developed for microneedle sampling [Publication I], microchip capillary electrophoresis [Publication II] [Publication IV] [Publication V] [Publication VI], electrical cell stimulation [Publication III], yeast cell filtering [Kolari 2010b], human cells culturing [Kolari 2009a] and DNA amplification [Hokkanen 2003].

### 3.1 Microneedle

A microneedle sampling system was developed for tissue sampling [Publication I]. The idea was to combine the high accuracy of silicon microneedle sampling to microscopy imaging with the aim that this system could be used in a hospital operating room. Such a set-up would allow a doctor to look at a microscope image of a tissue slice during a surgery and take microneedle samples for mass spectrometer (MS) analysis. For example, the microneedle would make it possible to extract the beginning of a new tumour without diluting the sample with a healthy tissue and the microneedle device itself would be disposable. The doctor could select for multiple extraction points and an automated robotic system would pick a new clean device for every slice extraction.

Takats et al. have also investigated so-called “Intelligent knife” device concept [Takats 2012]. Their device functions by heating a cancer tissue sample during surgery to create a vapour that is passed to a mass spectrometry. Cutting blade is embedded to 1/8” stainless steel tubing with 1/8” Teflon suction tube at the other end [Balog 2010]. Venturi air jet pump was used to pump the vapour sample to MS with nitrogen gas. The main difference between these two approaches is the vaporised sample, as the system outlined here uses a liquid-liquid extracted sample. Both analyzes are based on phospholipids which act as biomarkers for cancer [Schäfer 2009][Balog 2010][Hilvo 2011]. Cancer makes changes to a lipid metabolism of cell membranes already in early stage and it will alter the distribution of lipids. A single lipid is not a biomarker for a cancer. The distribution of cancer specific lipids is needed to measure and it is different for different tissues. Schäfer et al. and Balog et al. investigated lipid distribution changes in porcine, canine and rat. Hilvo et al. studied lipid profiles of breast cancer. This sampling system is more accurate, because it uses microneedles and microscopy for sampling whereas the Takats et al. system is more robust as
it does not use microscopy or any fluid handling. Moreover, the “Intelligent knife” is faster, because it able to give a result in few seconds. In contrast, although the microneedle system has automated robotics for lipid extraction, the analysis time is a few minutes per sample.

The author designed and modelled the silicon microneedle structure. Microneedles were about 200 μm high and two variants were tried, with 150 μm and 250 μm outer diameters. The PMMA chip acted as a closed microfluidic reservoir, which prevented contamination of the measurement system with cancer tissue. The PMMA chip possessed a 10 μL microfluidic channel for an extraction solvent and a pneumatic actuation chamber with TPU membrane. The microneedle had two fluidic ports that allowed simultaneous injection and sampling possible - a prerequisite for lipid sample extraction.

The operating principle of a blunt microneedle is presented schematically in Figure 34. Solvent is pumped from the upper left channel to the microneedle tip, which has a 30 μm deep chamber in contact with the tissue sample. Finally, the extracted sample is aspirated to upper right channel for MS analysis.

![Figure 34. Schematic cross-section of a silicon microneedle. Adapted from [Publication 1].](image)

The microneedle was tested by using it to extract the breast cancer biomarkers Phosphatidylcholine (PC) and phosphoethanolamine (PE) lipids from tissue samples [Hilvo 2011]. The tip of the microneedle is shown in the Figure 35a and an extracted tissue sample is in Figure 35b. As can be seen the tissue morphology was unmodified - as it should be - and only the lipids were extracted.
Microfluidic devices and fluorescence measurement systems

Figure 35. a) A silicon microneedle tip with 250 μm outer diameter. b) A breast cancer tissue sample after the microneedle extraction. Adapted from [Publication I].

The author also developed the microfluidic instrumentation for the extraction process. In this case, the challenging and highly volatile methyl tert-butyl ether (MTBE) solvent was used in this work as it is the only solvent that can extract PC+PE lipids without dissolving the PMMA chip [Publication I]. The first version of the polymer microfluidic part was just 1 μL but this proved to be too small, so a second 10 μL version was fabricated that made it possible to operate with MTBE. Different extraction times were tested from 1 to 8 min and it was found that the shortest (1 min) gave the best results. Further optimization is required including the development of a feedback loop for the flow velocity of the MTBE extraction buffer to the pneumatic pressure controllers. MTBE modified the PMMA polymer surface so that the constant flow velocity was not easily achieved. In addition, the elasticity of the TPU membrane decreased in repeated pneumatic actuation, which also may change the flow velocity of the solvent. The difference between the maximum and the minimum velocities was observed to be about ten-fold. A feedback loop from flow measurement to pressure controllers should stabilize the solvent flow.

The extracted sample was collected in a MS standard interface glass vial with a steel needle and an extra 10 μL of MTBE was added to ensure there was sufficient liquid for the MS. This means the sample was diluted in a 1:1 ratio, but this also prevented MTBE evaporation. PC and PE lipids were analysed from MS results with MZmine 2 software [MZmine]. The results of this analysis of PC and PE biomarkers in normal and in five tumour samples are shown in Figure 36. All these samples were from the same patient and from the same tissue sample, but they are from different positions. These samples were classified by a pathologist and threshold for tumour samples was taken to be more than 40% tumours cells, whereas normal tissue were tumour cell free. Samples were frozen at -80°C during surgery and thawed prior to measurement. The results clearly show increased PC and PE values in tumour samples and the signal variation in tumour samples probably comes from the different spatial extraction positions, as microneedle extraction is highly site-specific and the tissue sample did not have a constant lipid distribution.
Microfluidic devices and fluorescence measurement systems

Figure 36. MS results from microneedle extracted samples: measurement 0 is normal sample 14N and measurements 1-5 are tumour samples 14T. Adapted from [Publication I].

The microneedle used had two fluidic channels for simultaneous liquid injection and aspiration in a similar fashion to the microfluidic probe (MFP) used by Delamarche et al. [Lovchik 2012]. Both devices were made on silicon and microfluidic channels were in the order of a few hundred μm. In contrast the MFP was used for tissue staining, which required the presence of an immersion liquid between the tissue and the MFP. This immersion liquid provides the seal for simultaneous injection and aspiration whilst preventing the flooding and evaporation of the staining liquid. In our case, the microneedle tip (Figure 34) had a 30 μm deep flow chamber for lipid extraction and it was the edge of this chamber when pushed towards tissue that provided the required seal [Publication I]. Overall, this microneedle is not suitable for tissue staining, because it cannot withstand lateral movement, whereas the MFP is not suitable for lipid extraction as it also aspirates the immersion liquid.

Droplet based PDMS probe has been developed by Chen et al. who used it to measure insulin secretion from a single murine islet of Langerhans cells [Chen 2008a]. This work utilized simultaneous injection and sampling in a 30x20 μm² wetting spot. Moreover, they also demonstrated two parallel wetting spots with 15 μm spacing using fluorescence labelled liquids, however for the insulin secretion experiments only a single wetting spot was used. The fluidic system was based on droplets: glucose buffer was used to stimulate cells and the resulting insulin secretions were measured from the same sample droplets. This microfluidic probe system is, however, not suitable for lipid extraction from cancer tissues as the PDMS is not compatible with the MTBE solvent [Publication I]. In addition, our microneedle device had a closed liquid volume which was required for cancer tissue samples to avoid contamination of measurement system, but Chen had continuous droplet flow from pump to analysis. The microneedle could potentially be used for the insulin secretion application, but it does not provide as a good seal as soft PDMS probe. Sealing is also a challenge in lipid extraction and exact fluid flow control is needed.
Multifunctional PDMS pipette for single cell electroporation with NG-108-15 mouse neuroblastoma cells has been presented by Ainla et al. [Ainla 2012]. This PDMS pipette had continuous injection and pumping in open liquid volume, which is not applicable for the cancer tissue application as the MTBE extraction buffer is highly volatile [Publication I]. Additionally, the PDMS material is not compatible with extraction buffer. In contrast, the microneedle is not suitable for electroporation as it did not have an electrode like PDMS pipette, which incorporated Field’s metal as electrode into pipette tip using a metal filling method. Channel cross-sections for electrode filling and microfluidics were about 20x20 μm², whereas the size of the needle tip was about 200 μm.

3.2 Microchip capillary electrophoresis

Our microchip CE was realized in silicon-glass and SU-8 polymer versions with the aim of producing a high-speed microanalytical system with parallel CE analysis from the same sample. The author designed and modelled the CE chips [Publication IV] [Publication V]. The author also developed the microfluidic instrumentation and fluorescence measurement systems for the CE chips [Publication II] [Publication IV] [Publication V] [Publication VI].

The silicon-glass CE chip used immunoaffinity SPE for sample concentration (Figure 37). Moreover, the CE chip had a 1 μL SPE chamber, a 25 mm long separation channel and incorporated amorphous silicon thin film electrodes for high voltage (HV) and conductivity detection.

![Figure 37. Silicon-glass CE microchip [Publication IV].](image)

The author made the finite element method (FEM) simulations for the microfluidic flows and electrical fields [Publication V]. The microfluidic flows are shown in Figure 38 and the flow velocity was 1 mm/s in all simulations. As can be seen in Figure 38a, the pillar filter with 30 μm pillars and 25 μm spacing makes the fluid flow more uniform across the chamber. In addition, Figure 38b shows how the presence of 40 μm beads spreads the flow to whole SPE chamber – the pillar filters kept the beads in the chamber whilst the beads captured the sample. N.B. normally the chamber was full of beads, but it was not possible to create a simulation with a totally filled chamber.
The electric fields are shown in Figure 39. In Figure 39a the electric field is almost constant at the chip surface level, but in the cross section the highest electric field is observed near HV electrodes in the lower left corner and beginning of CE channel in upper right corner in Figure 39b. The SPE chamber was 400 μm high, as a result of the 40 μm size beads used in the measurements and the volume of the SPE chamber was significantly larger than that of the CE channel. Only the sample near CE channel was injected to separation, because the highest electric field was present in that area.

Our aim was to develop a microfluidic multichannel SPE-CE chip with immunoaffinity sample concentration [Publication IV]. An almost similar kind of immunoaffinity SPE-CE system with glass capillary fibers, but with much smaller SPE chambers has also been developed [Guzman 2003] [Guzman 2008]. The smaller chamber means more accurate injection and better separation performance. The smaller chamber enables also higher electrical field and a more effective injection. We should also have a smaller SPE chamber and separate injection channel from SPE to CE, however, on-chip valve development is needed first to allow separate injection channels. Moreover, we have not found that microfluidic multichannel immunoaffinity SPE-CE would have been demonstrated [Pagaduan 2015] [Nuchtavorn 2015].

On the otherhand single channel immunoaffinity sample capturing for microchip CE has been shown by Sun et al. who developed PMMA chip for protein measurements [Sun 2008]. The microfluidic channels were 100 μm wide and 10 μm high and UV photopolymerization was used to generate a 2 mm long immunoaffinity column. Human serum albumin (HSA) protein with fluorescein
Isothiocyanate (FITC) label for fluorescence detection was measured by first capturing the FITC-HSA sample to the 2 nL immunoaffinity column, followed by sample was eluting to CE separation. Their CE part comprised of a direct injection cross with a 100 μL volume and an approximately 2.5 cm long separation channel compared to our CE microchip which had a 1 μL immunoaffinity chamber and a 2.5 cm long separation channel [Publication IV]. Our chip should also have a separate injection channel between immunoaffinity chamber and CE channel like Sun et al., because it will give a more accurate injection to CE separation. Additionally, Sun et al. had a photopolymerized column and we had agarose beads for immunoaffinity capturing. The photopolymerized column is better for accurate injection when compared to agarose beads, however, the beads are better for immunoaffinity preparation as it could be performed under optimal environment without the microchip.

Immunoaffinity sample capturing for microchip CE has been used by Wellner and Kalish to measure four hormones from whole blood, saliva and urine samples [Wellner 2008]. The hormones examined included follicle-stimulating hormone, luteinizing hormone, testosterone and thyroid-stimulating hormone. A cross-shaped borofloat glass CE chip with microfluidic channels of 50 μm wide and 20 μm deep was used. In addition, the separation channel was 75 mm and the injection cross had a double-T shape with a 100 μm center-to-center distance. Commercial glass fiber filters (AP-40 from Millipore) were used as immunoaffinity inserts in fluidic connector by punching 2 mm disks from the 0.475 mm thick filters. It was possible to coat these filter inserts outside the microchip as we did with agarose beads [Publication IV]. Wellner and Kalish also had a separate injection channel from immunoaffinity filter to CE, which should be added to our system. It is also possible to microfabricate porous structures on chip for sample capturing and pillars, grass and three dimensional structures have all been demonstrated with silicon [Franssila 2010d]. These porous structures are under development for glass and polymer materials however and at least high aspect ratio pillars still remain a challenge for both materials.

3.2.1 LIF detection for microchip CE

A hot embossed PMMA chip that combined isotachophoresis (ITP) and zone electrophoresis (ZE) was used to measure β-blockers in [Publication VI]. The author developed LIF-measurement setup required for the analysis (Figure 40). A 488 nm argon laser (4 mW, Laser Graphics) was used for excitation and a cooled CCD camera (Hamamatsu Orca ER) for detection. The laser was filtered with a 488±5 nm bandpass filter and the excitation light was directed at about 45° to avoid laser light scattering to CCD. Laser was focused to a 200 μm spot through the 200 μm thick PMMA lid. Two bandpass filters 520±5 nm were used for fluorescence light filtering and the CCD camera had 10-50 ms exposure time for detection.
Conductivity detection was also used concurrently with LIF and a conductivity detector was built according to details outlined in Everaerts et al. [Everaerts 1976]. Conductivity and fluorescence were measured from oxprenolol and acebutolol separation at the same time as shown in Figure 41. Sample concentrations were 10 and 25 μg/mL for oxprenolol and acebutolol, respectively. Timescales of conductivity and fluorescence setups were not synchronized as they were typically measured separately. Total analysis time for ITP and CE on microchips was found to be 20 min. The LIF detection is an especially good for separation method development as you can select the detection position freely which is not possible with fixed conductivity detection electrodes. It was also shown that ITP preconcentration is suitable before β-blockers separation. Overall these results were the first time that any drugs from urine samples were measured using a PMMA ITP-CE chip.
Figure 41. Simultaneous a) conductivity and b) fluorescence measurement at same CE channel. Timescales were not synchronized to each other. Adapted from [Publication VI].

The same LIF-detection for microchip CE was also used with SU-8 CE chips (Figure 42). This epoxy polymer based chip had a solid phase extraction (SPE) chamber for sample concentration [Publication V]. In this case, the laser beam (40 mW, Laser Graphics) was divided (50%/50%) with a beam splitter: the first beam was focused to 100 μm spot into the CE channel and the excitation light angle was again 45°. The second beam was coupled to the SPE chamber without focusing in order to measure the whole SPE chamber. This second beam was attenuated with a bandpass filter (488±1 nm) to produce fluorescence signals at the same level. Moreover, it was possible to measure sample concentration in SPE chamber and CE separation with the same CCD camera which had a 5 ms exposure time for detection. The measurement window was 50x50 μm² and 1x1 mm² at the CE channel and at SPE chamber, respectively. Laser intensity was the main difference in the fluorescence measurement at CE channel with PMMA and SU-8 chips. The background fluorescence from the SU-8 was higher and therefore ~20x more laser intensity was needed (PMMA chip: 4 mw at 200 μm spot, SU-8 chip: 20 mW at 100 μm spot).
The same measurement system was used again with a silicon-glass CE chip setup [Publication IV]. This time the interface for microfluidics and electrical contacts was implemented (Figure 43). The CE chip had a groove for a capillary pipe connection and the measurement interface had valves for fluidic control. The Silicon-glass chip had amorphous silicon thin film electrodes for electrical contacts, whilst the measurement system possessed electrical connection needles for HV and conductivity detection. The silicon-glass chip was first tested without sample concentration (Figure 44a) and black A curve is the sample concentration with SPE and the gray B curve is the measurement result in the CE channel. Silicon-glass and SU-8 chip were both used for immunoaffinity based SPE in Figure 44b and 44c, respectively. The sample was FITC labelled testosterone with concentrations 2.5 ppm, 1 ppm, and 2.5 ppm (Figures 44a-c), respectively. As can be seen the presence of immunoaffinity based SPE increased signal level in the CE. When the results were analysed, the SU-8 showed a higher fluorescence background compared to the others and the silicon-glass chip was found to be between 10 to 100 times more sensitive.
Figure 43. Microfluidic and electrical contact for CE chip measurements [Publication IV].

Figure 44. Immunoaffinity-based SPE sample concentration and CE separation with FITC-la- beled testosterone. a) Separation without immunoaffinity SPE on silicon-glass chip, b) silicon- glass chip and c) SU-8 chip. Measured curves A are sample concentrations in SPE chamber and curves B are CE separation results in the end of the CE channel (25 mm from SPE chamber). Adapted from [Publication IV].

In our collaboration with the Mathies’ group a disposable R2R PMMA CE chip and a confocal four colour LIF-detector were developed for R2R PMMA chips (Figure 45a) [Publication II]. The Mathies’ four colour LIF detector (in Figure 26) was modified for use with a PMMA CE chip - there was only one CE channel and rotating detector was not needed. The thin 250 μm thick polymer chips also
required a change to the optics of the measurement system as common microscope objectives with short working distance were now possible and the confocal parts also were modified. The same type four colour Hamamatsu’s PMT was used. It was found that a lower laser excitation intensity of 18 mW was sufficient in this antibiotic resistance gene mecA application, because the light collecting efficiency was better for the thinner polymer chips. The separated peaks comprised of mecA 212 base pair (bp) (peak 2) and gyrB 491 bp (peak 4) gene fragments from Staphylococcus epidermidis (Figure 45b). DNA size ladder was 100 (peak 1), 250 (peak 3) and 600 (peak 5) bp. Sample concentrations were 1 ng/mL per each fragment and the sample was stained with 1:10 000 (v/v) diluted SYBR® Safe fluorescence label. Both PMMA CE chips [Chen 2008b] and hot roller embossed PMMA CE chips [Ng 2009] have been previously demonstrated, however, these R2R PMMA CE chips are the first disposable and mass manufacturable device. Moreover, the presence of the thin PMMA foil has an additional benefit for the fluorescence detection.

Figure 45. a) Confocal four colour LIF-detector for R2R PMMA based CE chips. b) CE separation of mecA positive PCR sample. Adapted from [Publication II].
3.3 Cell chips

3.3.1 Electrical cell stimulation

Electrical measurements were developed for hERG-CHO-K1 hamster cells and human embryonic stem cells [Publication III]. The author participated in the development of the titanium (Ti) microelectrodes on glass microchips. The Ti electrode had a width of 40 μm with a spacing 80 μm. Ti oxidises rapidly at room atmosphere to produce a 5-10 nm titanium dioxide (TiO$_2$) surface, capacitive coupling from Ti to the incubation medium was efficient. In addition, the cells also adhere to electrodes and TiO$_2$ dielectric layer prevents electrolysis. The stimulation system was first developed with CHO cells using a voltage sensitive fluorescence label (FLIPR membrane potential assay, Molecular Devices Inc.) and it was possible to detect ion channel activation with fluorescence measurement. FLIPR dye will give increased fluorescence signal, when it binds intracellular lipids and proteins. The potassium K1 ion channel of CHO cell was activated by square waves with a 0-7 V$_{pp}$ amplitude and the stimulation frequency of square waves were 500-1000 Hz with 30 pulses. It was found out that fluorescence signal increases as a function of the stimulation amplitude. Signal was fully depolarized with 7 V$_{pp}$ amplitude and it was verified by KCl depolarization, which did not further increase the fluorescence signal.

Next human embryonic stem cells derived cardiomyocytes were stimulated with Ti-TiO$_2$ electrodes, as shown in Figure 46. It was found that a reduced amplitude of 4 V$_{pp}$ was sufficient for long-term stimulation of the cardiomyocytes, though the same stimulation frequency and pulse number could be used. In addition, the native beat cycle altered from about 1 s to 800 ms with stimulation.

![Figure 46](image)

Figure 46. Human embryonic stem cells-derived cardiomyocytes on Ti-TiO$_2$ electrode array from [Publication III].

Cardiomyocytes were stimulated on microelectrodes by Nishizawa et al., who used a polyimide coated glass chip with sputtered platinum electrodes [Nishizawa 2007]. The electrodes were 20 μm wide and with 10 μm spacing with electrically conductive polymer polypyrrole (PPy) deposited on the surface. PPy has a strong affinity for both the platinum and the polyimide support, which was modified with self-assembled alkylsilane monolayer. Cardiomyocytes were cultured on the microelectrodes and loaded with 10 μM fluo-3 AM (Molecular
Probes) fluorescence labelled calcium Ca\(^{2+}\) indicator. Electrical stimulation releases cytosolic Ca\(^{2+}\) and increases fluorescence signal. The cells were stimulated with negative current pulses: interval 1 s, duration 100 ms and amplitude 10 mA and it was possible to measure cellular responses to the electrical stimulation with fluorescence measurement. We also stimulated cardiomyocytes, but we did not have a fluorescence label to detect electrical response of cardiomyocytes [Publication III]. Nishizawa et al. had a single cell layer of cardiomyocytes on the electrode surface, in contrast our cardiomyocytes formed clusters (see Figure 46). Additionally, we monitored beating cycle of cell clusters, whereas Nishizawa et al. concentrated on measuring the electrical response over cell layer. Their results showed a threshold charge for myocyte stimulation of 0.2 \(\mu\)C with 0.8 cm\(^2\) cell layer, which meant about a 250 mV membrane polarization.

Electrical cell stimulation of nerve cells has also been performed by Fromherz who examined the nerve cells of a pond snail on a silicon CMOS chip [Fromherz 2008]. He used an electrolyte-oxide-semiconductor (EOS) capacitor for stimulation and an EOS field-effect transistor (FET) for detection. The EOS stimulation activated cell membrane ion channels: the sodium channel Na\(^+\) was the inward and the potassium K\(^+\) the outward ion channel at cell membrane. A rising voltage ramp of +85 mV/ms activated the Na\(^+\) channel and a falling voltage ramp -85 mV/ms activated the K\(^+\) channel. Our chip did not have EOS-FET for detection, instead we used a voltage sensitive fluorescence label for ion channel detection. Although voltage sensitive labels are easy to use, there are not fluorescence labels for all ion channels, whereas EOS-FET detection is more universal and it gives ion voltages directly. Fluorescence labels require the calibration of fluorescence signals in order to get voltage values. Fluorescence method has also some other drawbacks. Sample preparation for fluorescence labels is needed before measurements. Fluorescence method is also invasive and this might have some effect for the results. In addition, photobleaching is one possible problem, where fluorescence signal decreases because of a high excitation intensity and a long excitation time.

### 3.3.2 Cell filtering

The author designed a yeast cell filter for an on-line bioprocess measurement [Kolari 2010b]. Silicon is a good material for this application as it tolerates the harsh washing steps used in the on-line measurement. In addition, hot water steam and alcohol/solvent washing steps are possible with silicon filters. The silicon chip used had 480 filter membranes and the diameter of each membrane was 150 \(\mu\)m (Figure 28a). One filter membrane contained about 3000 holes with 2.5 \(\mu\)m spacing in a honeycomb structure (Figure 28b). The diameter of the holes was 750 nm after silicon etching, though this hole size was decreased with thermal oxidation down to 350 nm and overall the total number of holes was over 10\(^6\). The maximum operating pressure was 5-7 bar for silicone rubber gaskets, whilst a 10 \(\mu\)m thick SOI membrane will theoretically tolerate a pressure difference of several hundreds bar across the membrane. The filters were tested with 11 mg/mL yeast cell solution and it was found that washing steps were
needed after a few microliters of sample, because bioprocess sample had lots of extracellular material. Normal flow rate were 1 μL/s.

Silicon microsieve has been fabricated on SOI wafers by Lim et al. [Lim 2012]. The diameter of their silicon chips were 7.5 mm and diameter of the filter area 5 mm. Each filter had 10^5 holes with 10 μm diameter and 15 μm spacing with the whole filter area supported by honeycomb rings with a 780 μm diameter. In addition, the holes were within the honeycomb structure. Lim et al. filtered circulating tumour cells by size with their silicon filter using a 1 ml/min flow rate and fluorescence microscope for cell counting. Pressure drop through the filter membrane was found to be just a few mbar. They spiked fluorescence labelled MCF-7 breast cancer and HepG2 liver cancer cells into human blood and achieved a recovery rate >80% for the microsieve filtering. Overall, the Lim et al. filter has too large holes for HEPA filtering, whereas our filter’s holes were too small for a 1 mL blood volume and would have clogged with blood cells [Kolar 2010b].

Silicon nitride (SiN) membrane filters have also been used in bacteria measurements by Nguyen et al. [Nguyen 2015]. They used 2, 3.5 and 5 μm size filters for to detect Salmonella bacteria which are typically ~0.7 x 2 μm². The bacteria capturing was based on an antibody coating: the first tests with no coating allowed all the bacteria to pass through, however when the antibody coating was applied filter efficiencies were 52, 30 and 12% with 2, 3.5 and 5 μm microsieves, respectively. The bacteria suspension comprised of 10^7 cfu/mL and flow rate was 16 μL/(min-mm²). Microfiltration with hole sizes smaller than bacteria leads to biofouling problems and moreover, it is difficult to filter large mL volumes. Use of an antibody coating and larger holes than bacteria make the filtering of mL volumes possible. Our HEPA filter also had too small holes for Salmonella bacteria application and the large 1 mL sample volume would have clogged the holes of our filter. The filters of Nguyen et al. also have too large holes for HEPA filtering and although their filters could potentially capture yeast cells, bioprocess samples have lots of small particles and a smaller filter size is needed.

It is possible scale up our filter 2-5 μm size (to match Nguyen’s filter) and to develop an antibody coating for it. It is also possible to scale down the Nguyen filter design to match our 350 nm size. However, isotropic reactive ion etching (RIE) is used for SiN and membrane thickness should be smaller than the hole size to ensure narrow hole distribution, which means about 100 nm thick SiN membrane with 350 nm holes. Thicker nitride is also not an attractive option because of high film stresses. We demonstrated a filter with 350 nm holes in 10 μm thick SOI (aspect ratio of ~30:1), which means that SOI filter membrane could be about 100 times thicker than SiN membrane. SiN has a slightly better Young’s modulus and yield strength (300 GPa and 14 GPa) than silicon (190 GPa and 7 GPa), but the thicker SOI membrane is much stronger than SiN membrane [Franssila 2010e] [Franssila 2010f].
3.3.3 **Cell culturing**

The author designed and modelled microfluidic chips for cell culturing and a schematic of the glass-silicon microchip used for cell culturing is presented in Figure 47a [Kolari 2009a]. This cell chip was designed for long term measurements and to prevent cross contamination between cell spots. Prostate cancer cells were cultured in hydrophilic circles present on the glass surface (Figure 30). The medium flow channel for 40 cell samples was developed on a silicon chip (Figure 47b) and the channel system was designed so that there is no flow between cell spots. Moreover, it is also possible to coat the cell spots with different drugs without cross contamination. In addition, the author used FEM modelling for the microfluidic channel design (Figure 47c) and these were designed for 1 mm/s flow velocities (2-4 μL/min).

![Schematic glass-silicon microchip for cell culturing.](image)

*Figure 47. a) Schematic glass-silicon microchip for cell culturing. b) Silicon microchannel for medium feeding. c) FEM modelling of medium flow. Adapted from [Kolari 2009a].*

In the measurements prostate cancer cells were first cultured on the glass slide. Next the glass and silicon parts were clamped together and flow with Sigma DMEM D5546 cell culturing medium was started. Both the glass and silicon surfaces had a hydrophobic coating at contact surfaces which provided a good seal with only a small clamping force.
Our cell culturing chip bears a resemblance to the PDMS chip of Hung et al. shown in Figure 20 [Kolari 2009a] [Hung 2004]. Both had medium flow channels for long time cell culturing in an array of microfluidic cell chambers and our system worked with 2-4 μL/min velocities, whilst their system used 0.1-1 μL/min. We have 4x10 chamber array and they have 10x10 chambers. Our device had 300 μm deep chambers with about 400 μm diameter and they have 40 μm deep chambers with 1 mm diameter. Chamber volumes are almost the same 30-40 nL. Both systems had passive liquid couplings, which are flow velocity dependent. A lower medium flow rate might be better also for us, but those passive liquid couplings should be redesigned for the lower flow rate. Our system, featured clamping, which made cell and drug spotting possible, whereas the Hung et al. system was closed preventing spotting. Their chip was designed for concentration gradient generation from two liquids for cell based assays, in contrast, our system was for drug screening. With our system there was no possibility of cross contamination possibility, whereas their system possessed medium flow through several chambers.

Cell culturing microchambers have also been developed by Akagi et al. [Akagi 2004]. Each chamber was 550x550x250 μm³ and the total number of chambers was 1248 in a 2.54x7.62 cm² area. These 50 nL microchambers did not have microfluidic medium feeding channels and all liquid handling was performed with dispensing robots. They are simple and easy to use, but long term measurements are more difficult and cross contamination is expected. Akagi’s static fluidics chip, however, fails to emulate many important in vivo phenomenon, like shear stress, and therefore fluidic cell growth systems are important. Our chip is capable of emulating in vivo phenomenon without the complication of cross contamination.

3.4 DNA amplification

DNA amplification on a silicon microchip was developed [Hokkanen 2003]. Amorphous silicon and molybdenum thin film heater and thermistor elements were deposited in the bottom of a silicon chip (Figure 48a). The size of silicon chip was 8x12 mm² and it had one or two 1-10 μL liquid chambers, which were 350 μm deep (Figure 48b). In addition, the silicon surface was oxidized in order to prevent inhibition of the silicon surfaces [Kolari 2008a].

![Figure 48](image-url)

**Figure 48.** a) Schematic PCR chip structure. b) Optical photo of silicon PCR chip. From [Hokkanen 2003].
The In-Check system was used by Petralia et al. [Petralia 2013]. This silicon PCR chip had two 12 μL chambers for sample preparation and DNA amplification. Sample preparation included single step DNA extraction from cultured cells or from whole blood and DNA amplification was performed in the same chamber. After sample preparation and DNA amplification the sample was detected with a microarray in the separate chamber. Human β-globin gene was measured from cultured human ovarian cancer cells and from whole blood. The novelty of this system is that it incorporated fully on-chip integrated DNA extraction and amplification with microarray detection at the end. The whole lab-on-chip process takes less than 2 hours and efficiency was found to be better than with common laboratory equipments. Our PCR chip had also two silicon chambers for DNA amplification, but it did not have microarray chamber for detection [Hokkanen 2003]. We used real-time fluorescence measurement for detection and had capillary connections to reaction chambers, which makes it possible to take the sample for separate analysis after amplification. In-Check PCR chip had higher heating rate 40°C/s but lower cooling rate 10°C/s than our chip as the maximum heating and cooling velocities of our silicon PCR chip were 20-30°C/s.

Silicon PCR chip was also presented by Min et al. [Min 2011]. They used silicon pillar packed chip for DNA purification to capture Escherichia coli bacteria from blood samples. They had kosmotropic salt based chemistry, which generate strong water molecule structure on the hydrophilic surface and DNA binds indirectly to this hydration layer trough hydrogen bonding. Pillars had a cross-section of 25x25 μm² and a height of 100 μm with 15-17 μm spacing. The chamber was also 100 μm high and had a volume of 3 μL. We used also pillars, but our pillars were to enhance heating velocity, which is based on increased surface-to-volume ratio. We tested 100x100 μm pillars with 50-100 μm spacing and determined that pillars increased the heating rate by a few °C. We also found the same problem with pillars as Min i.e. that pillars increase PCR inhibition because the surface-to-volume ratio is increased. Min solved this problem by adding 5% (volume/volume) polyethylene glycol (PEG) surfactant. Moreover, pillars also enhanced the filling of the PCR chamber as the pillars increases capillary force within the chamber. Pillars also had one drawback for real-time fluorescence detection, because they increased excitation light scattering and the need for filtering. We did not use pillars in the final measurements because of those inhibition and scattering problems and we did not have on-chip sample capturing.

Silicon pillars have been also used to improve temperature uniformity as shown by Ibrahim et al. who modelled and fabricated three different silicon pillar chips for experiments [Ibrahim 2014]. They made 2-3 μL size chambers which were 300 μm deep and had 115, 157 and 246 μm pillar diameters with 15x15, 11x11 and 7x7 pillar arrays, respectively. The largest pillar array had the best temperature behavior and they found out that the 15x15 pillar array decreased the temperature dispersion by 84%. The main drawback of this decreased temperature
dispersion was the increase in surface-to-volume ratio which was 18 mm$^{-1}$ for the 15x15 pillar array. Surface area is about two times higher with pillars. They tested PCR amplification efficiency with 148 bp fragment sample from blood and showed a successful amplification result using slab gel electrophoresis with the 15x15 pillar chip.

3.4.1 Fluorescence detection for DNA amplification

The author developed real time LIF measurement setup for silicon PCR chips [Hokkanen 2003] shown in Figure 49a. Excitation power of argon laser was attenuated so that the effective intensity in PCR chamber was about 0.1 mW - showing that LED excitation would also be possible. PCR amplification was demonstrated on the silicon chip, Figure 49b. A SYBR Green fluorescence label was used and it was diluted to 0.1x concentration from the original 10 000x and DNA concentration was 2 ng/μL (4·10$^7$ copies/μL). Temperature cycling had three ramp levels: 94, 60 and 72°C. The SYBR Green label gives signal only when it is bound to a double-stranded DNA. Depending on a temperature there is double-stranded or single-stranded DNA and the fluorescence signal is changed in Figure 49b. The signal increased by 10-15% per cycle, before PMT signal saturated (typically PCR amplification saturated after 20 cycles).

![Real time LIF-measurement setup for PCR chips](image)

![DNA amplification on microchip PCR](image)

**Figure 49.** a) Real time LIF-measurement setup for PCR chips. b) DNA amplification on micro-chip PCR. Adapted from [Hokkanen 2003].
A miniaturized fluorescence detector for PCR was presented by Rafał Walczak [Walczak 2011]. He had a red diode laser 636 nm with 1 mW intensity for fluorescence excitation and a non-cooled CCD for detection. The detector was hand-held, portable, and had a power consumption of less than 2.5 W. Campylobacter was measured from chicken faecal samples and showed a sensitivity of 0.7-7 ng/ml from a 2.5 μL PCR chamber. Our fluorescence detector was a more laboratory-based setup that used a 40 mW 488 nm laser to produce a 0.1 mW excitation power. Overall, Walczak had a 10x higher excitation intensity, but a less sensitive detector than our PMT.

A fluorescence detector for droplet array PCR was demonstrated by Yu et al. who developed a LED-based excitation with a non-cooled CCD camera [Yu 2011]. They used a 6x6 (10x10 mm²) array with 500 nL droplets and blue LEDs with a 3 mW intensity [Zhang 2011]. The detector was built on a commercial thermal cycler, but silicon PCR chips were used for the measurements. Mir-122 ribonucleic acid (RNA) from mice was measured and it was found that PCR amplification was inhibited totally with a sample above 2340 pg per droplet. This is about the same concentration 4.6 ng/μL than we needed with our chip 4 ng/μL. They have less sensitive fluorescence detection system, because they used a smaller excitation light intensity 2 x 3 mW at 10x10 mm² area (~0.06 mW/mm²) and we had 0.1 mW at 0.1 mm diameter spot (~13 mW/mm²). In addition their non-cooled CCD is less sensitive than our PMT.
Small volume liquid handling and fluorescence detection were studied in this thesis. The author designed, modelled and measured microfluidic chips, which were developed on silicon, glass and polymer materials. Easy and repeatable measurements are a challenge for small liquid samples, therefore the author also developed instrumentation for microfluidic measurements. In practice this means fluidic, electrical and optical connections to microfluidic chips. Our applications are such that use of a small liquid amount brings some benefit when compared to existing laboratory equipments. New tools were developed for tissue sampling, DNA amplification, molecular separation and cell culturing, filtering and measurements. Fluorescence measurements were used in almost every application, because they are suited to measure low concentrations from small volumes. The author developed laboratory setups to demonstrate that the microfluidic chips were working.

The author worked on breast cancer sampling, where microneedle sampling was combined with the microscope imaging of cancer tissue samples. A new multi-port silicon microneedle structure was invented and developed. The microneedle had two separate channels in the same microneedle, which made simultaneous injection and sampling possible. Cell membrane lipids were extracted from 100 and 200 μm spots that were selected from a microscopy image, whilst an automated robotic system controlled microneedle operation and a mass spectrometer identified the phosphatidylcholine and phosphoethanolamine lipids, which are biomarkers for breast cancer. In the future this microneedle sampling system could be utilized in hospitals to help doctors with cancer diagnoses during surgery, but more development is still needed. The needle was demonstrated with preselected normal and tumour samples, but the next step is to use a tissue sample, which has both normal and tumour cells. Increased magnification is also needed for the microscopy system so that it is possible to identify potential sampling positions without a separate microscope. In addition, the microneedle sampling needs a liquid flow velocity measurement to feedback loop for the pressure controllers as the extraction buffer modifies PMMA chip surface and fluid resistance is decreased, which increases the flow velocity. Also the elasticity of the TPU membrane decreases with use in pneumatic actuation and changes the flow velocity. The feedback loop will stabilize velocity variation of the solvent and allow extraction time optimization.
We demonstrated cell culturing microchips and electrical cell measurements. Microfluidic channels were developed for long term cell culturing measurements and titanium stimulation electrodes were demonstrated with cardiac stem cells. Titanium electrodes are not typically used, because they self-oxidise in room atmosphere and insulating titanium dioxide layer reduce electrical contact. This was, however, beneficial in our stimulation experiments, because it made capacitive coupling possible, which prevented electrolysis and allowed higher stimulation amplitudes. There is still further need for development of in vitro cell culturing. The cell culturing environment should be closer to the conditions present in a human body, which means intelligent control for medium flows, physical and chemical environment. Several cell types are also needed in the same cell culturing system in order to emulate the organs in the human body. Such organs-on-a-chip type cell culturing systems will decrease the need for future drug testing on animals and humans.

Microfabrication is also a good technology for cell filtering because microfluidics makes cell filtering much faster and cheaper than with existing laboratory equipment. The author has developed microfluidic filters for agarose beads and yeast cells. The beads were used for sample concentration in testosterone measurements and yeast cells for bioprocess monitoring. In the future smaller chambers should be developed for agarose beads, because it enables higher electrical field and a more effective sample injection. Yeast cell application needs still development for sample preparation and filter with a clogging monitoring would make it easier.

We have developed microchip immunoaffinity solid phase extraction (SPE) capillary electrophoresis (CE), where agarose bead sample concentration was used. One channel system was demonstrated both in silicon-glass and in SU-8 materials, whilst the final goal and novelty were in a multichannel SPE-CE from the same sample. This multichannel system would speed up analysis time in a doctor’s office, but a valving system is first needed for fluidic control in sample concentration and injection. In addition, a separate injection channel after SPE would make the sample injection more accurate.

We showed DNA amplification on silicon microchip, which had pillars in reaction chamber to enhance heating rate and temperature uniformity. We found out, however, that pillars increase DNA inhibition and make also fluorescence detection more difficult, because pillars increase surface-to-volume ratio and excitation light scattering. Finally we used reaction chambers without pillars and got also with this way fast 20-30°C/s heating/cooling rates. There is still one good reason to use pillars: sample capturing. Sample capturing and preparation need still more development and that should be a research focus in the future. Sample preparation should be on-chip. There is a benefit to use fast microchip DNA amplification outside of the laboratory environment. The possibil-
ity to combine all analysis steps; including sample preparation, DNA amplification and detection will increase applicability of the system. Microfluidics is a good technology to do this in a closed device.

Microfluidics technology is still mostly in research phase, but application development for microfluidics will be the focus in the future. There is already a reasonable amount of basic tools for demonstrators. Totally independent microfluidic devices will come, because point-of-care (POC) testing should be as simple as possible. POC testing will utilize more and more microfluidic chips that will provide huge savings to healthcare systems.

Next generation gene sequencing is one the most promising future application for microfluidics. Microfabrication tools are needed to identify genes in a fast and cost-effective manner. The next generation devices are based on electrical measurements, while earlier devices used CE with optical measurements. New microfabricated devices can increase the throughput and decrease costs. Moreover, it makes it possible to develop personalized medicine for healthcare. This would not just mean large savings, but would also lead to much improved treatment and a better quality of life for patients.
References

[3M] www.3m.com
[Abbott] www.abbottpointofcare.com
[Agilent] www.agilent.com
[Alere] www.alere.com
[Aquamarijn] www.aquamarijn.nl
[Axion] www.axionbiosystems.com
[Cellectricon] www.cellectricon.com


[Cytoo] cytoo.com

[Damme 2009] Pierre Van Damme, Froukje Oosterhuis-Kafeja, Marie Van der Wielen, Yotam Almagor, Ofer Sharon, Yotam Levin, Safety and efficacy of a novel microneedle device for dose sparing intradermal influenza vaccination in

[Debiotech] www.debiotech.com


[Dermaroller] www.original-dermaroller.de


[Dolomite] www.dolomite-microfluidics.com


[DropBot] microfluidics.utoronto.ca/dropbot


[EDC] www.edcbiosystems.com


[Engineering toolbox] www.engineeringtoolbox.com


[Fluidigm] fluidigm.com

[FPNotebook] www.fpnotebook.com/hemeonc/lab/RdClDstrbtnWdth.htm


References

[Gray 1697] Stephen Gray, A letter from Mr. Stephen Gray from Canterbury, May the 12th 1697, concerning making water subservient to the viewing both near, distant objects, with the description of a natural reflecting microscope, Philosophical Transactions, 1697, 19, 539-542.


[Illumina] www.illumina.com

[Innosieve] innosieve.com


[Labcyte] www.labcyte.com


[Li 2015] Hairui Li, Yong Sheng Jason Low, Hui Ping Chong, Melvin T. Zin, Chi-Ying Lee, Bo Li, Melvina Leolukman and Lifeng Kang, Microneedle-Mediated Delivery of Copper Peptide Through Skin, Pharmaceutical research, 2015, 12 pages, DOI: 10.1007/s11095-015-1652-z.

[Life Technologies] www.lifetechnologies.com

[Lim 2012] Li Shi Lim, Min Hu, Mo Chao Huang, Wai Chye Cheong, Alfred Tau Liang Gan, Xing Lun Looi, Sai Mun Leong, Evelyn Siew-Chuan Koay and Mo-Huang Li, Microsieve lab-chip device for rapid enumeration and fluorescence in situ hybridization of circulating tumor cells, Lab on a Chip, 2012, 12, 4388–4396, DOI: 10.1039/c2lc20750h.


[Microfluidic-chipshop] microfluidic-chipshop.com

[MicroHyla] www.cosmed-pharm.co.jp

[Micronit] www.micronit.com


[Min 2011] Junhong Min, Joon-Ho Kim, Youngsun Lee, Kak Namkoong, Hae-Cheon Im, Han-Nah Kim, Hae-yeong Kim, Nam Huh and Young-Rok Kim, Functional integration of DNA purification and concentration into a real time micro-PCR chip, Lab on a Chip, 2011, 11, 259–265, DOI: 10.1039/c0lc00320d.


[Multi Channel] www.multichannelsystems.com


[MZmine] mzmine.sourceforge.net


References


[RainDance] raindancetech.com

[RBDW eMedicine] emedicine.medscape.com/article/2098635-overview#aw2aab6b2


[Shimadzu] www.ssi.shimadzu.com


[Sullivan 2010] Sean P. Sullivan, Dimitrios G. Koutsonanos, Maria del Pilar Martin, Jeong Woo Lee, Vladimir Zarnitsyn, Seong-O Choi, Niren Murthy,


References


[Varioptics] www.varioptic.com


[Veredus] vereduslabs.com


[Yu 2011] Zeqi Yu, Ying Zhu, Yunxia Zhang, Juan Li, Qun Fang, Jianzhong Xi, Bo Yao, Nanoliter droplet array for microRNA detection based on enzymatic stem-loop probes ligation and SYBR Green real-time PCR, Talanta, 2011, 85, 1760–1765, DOI: 10.1016/j.talanta.2011.06.075.


In this thesis microfluidic chips were developed for tissue sampling, molecule separation and electrical cell stimulation. Microneedle sampling was demonstrated in cancer tissue analysis. Phosphatidylcholine and phosphoethanolamine lipids were extracted as biomarkers for a breast cancer. Microchip capillary electrophoresis was used to test testosterone measurements and to an antibiotic resistance measurement with meCA gene. Human embryonic stem cells were stimulated with microelectrodes, which were deposited on glass chip.

Microfluidics makes fast healthcare analysis possible in doctor’s office or even in home without time-consuming laboratory analysis. Treatment and medication could be started immediately without couple of days delay also avoiding unnecessary medication. Microfluidic devices have a small sample amount and low reagent consumption, which means lower costs than laboratory analysis. This all means more effective treatment and big savings for healthcare system in future.