

Dissertation

Fluidic Microsystems for Biochemical Analysis

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Abstract

The concept of miniaturised total analysis systems (μ TAS) came up in the early 1990s. This approach implicates the integration of a (bio)chemical sensor into a microsystem combined with on-chip reagent processes and microfluidics. Such miniaturised analysis systems allow to carry out measurements from small volumes of complex fluids with high efficiency and speed.

In this thesis, a modular design of a self-contained, cost-efficient and miniaturised system for fast DNA analysis is presented. The system consists of an on-chip DNA amplification unit based on the PCR method and of a capacitive microsensor for label-free DNA detection. The DNA detection principle relies on a capacitance change at the surface of interdigitated sub-micron electrode pairs during the DNA hybridisation process caused by the accumulation of complimentary DNA strands. Fast on-chip DNA amplifications and the performance of the label-free sensing principle have been successfully demonstrated. Both devices are fabricated as separate chips, which are finally combined with a fluidic system allowing the reagent to be transported from one chip to the other. A portable control-measurement unit has been realised, which allows thermocycling at predefined temperature steps for the PCR and to measure capacitance changes on the surface of several electrode pairs in parallel. The combination of the PCR chip, the DNA sensor, the fluidic system and the control-measurement unit composes a cost-efficient and miniaturised analysis system, which is suitable for the amplification and detection of specific DNA sequences in a point-of-care format for biochemical analysis.

Further this thesis deals with the issue of microfluidics, which is a main research field of μ TAS. Here, the flow behaviour of a sample stream inside a microchannel is studied, which is focused by vertical and horizontal neighbouring sheath flows. This so-called hydrodynamic focusing technique offers the possibility to adapt the dimensions of the sample stream and its position inside the microchannel by varying the flow rate ratio between the sample liquid and the individual adaptable sheath liquids. Two microfabricated flow-cells have been realised and characterised; one for non-coaxial sheath flow (sample stream is surrounded at three sides, while at the fourth side the sample moves along the channel bottom) and one for coaxial sheath flow (sample

stream is squeezed on all four sides). Experiments have been carried out with both devices, where the size, shape and position of the sample stream have been established by laser scanning confocal microscopy and dye intensity analysis. In microchannels (typically $25 \times 40 \mu\text{m}^2$) the creation of sample stream diameters that were five to ten times smaller than the channel dimensions were achieved, and the smallest measured sample stream width was $1.5 \mu\text{m}$. The mechanism to control the sample stream inside the microchannel allows the sample dimensions to be adapted over a wide range. A comparison of the experimental results with fluidic simulations show excellent agreements. In combination with (integrated) sensors, these microfluidic devices are suitable for on-chip single cells analysis.

Nomenclature

0.1 List of Abbreviations

| Symbol | Description |
|----------------------------------|---|
| 16S rDNA | DNA coding (16S subunit of prokaryotic ribosomes) |
| 2D | two-dimensional |
| 3D | three-dimensional |
| A | adenine |
| AC | alternating current |
| ADC | analogue-digital-converter |
| Ag | silver |
| bp | basepair |
| BSA | bovine serum albumin |
| C | cytosine |
| CDC | capacitance-to-digital converter |
| CFD | computational fluid dynamic |
| C ₃ H ₈ O | isopropyl alcohol |
| C ₆ H ₁₂ O | methylisobutylketone |
| Cy3 | cyanine 3 |
| DC | direct current |
| DEP | dielectrophoretic |
| DI | de-ionised |
| DNA | deoxyribonucleic acid |
| dNTPs | deoxyribonucleotide triphosphates |
| DRIE | deep reactive ion etching |

| Symbol | Description |
|---|---|
| dsDNA | double-stranded DNA |
| DVD | digital versatile disc |
| FACS | fluorescence activated cell sorter |
| FEM | finite element method |
| G | guanine |
| GPIB | general purpose interface bus |
| H ₂ O ₂ | hydrogen peroxide |
| H ₂ SO ₄ | sulfuric acid |
| IC | integrated circuit |
| I ² C | inter-integrated circuit |
| kb | kilo base |
| KCl | potassium chloride |
| KOH | potassium hydroxide |
| LC | liquid crystal |
| LED | light-emitting diode |
| LPCVD | low pressure chemical vapour deposition |
| M | molar |
| MEMS | micro-electro-mechanical system |
| MgCl ₂ | magnesium chloride |
| Na ₂ S ₂ O ₈ | sodium peroxodisulfate |
| PBS | phosphate buffered saline |
| PCB | printed circuit board |
| PCR | polymerase chain reaction |
| PDE | partial differential equations |
| PDMS | polydimethylsiloxane |
| PEB | post exposure bake |
| PGMEA | 1-methoxy-2-propyl-acetat |
| PMMA | polymethylmethacrylate |

| Symbol | Description |
|--------------------------------|------------------------------------|
| POM | polyoxymethylene |
| Pt | platinum |
| PWM | pulse-width modulation |
| RIE | reactive ion etching |
| RTD | resistance temperature detector |
| rpm | rounds per minutes |
| rRNA | ribosomal ribonucleic acid |
| Si ₃ N ₄ | silicon nitride |
| SiO ₂ | silicon dioxide |
| SLR | single-lens reflex |
| ssDNA | single-stranded DNA |
| T | thymine |
| Taq | thermus aquaticus |
| US | ultrasonic |
| USB | universal serial bus |
| UV | ultraviolet |
| w/v % | weight/volume percentage |
| μC | microcontroller |
| μTAS | miniaturised total analysis system |

0.2 List of Constants

| Constant | Description | Unit |
|-----------------|-------------------------|--|
| α | temperature coefficient | $3.85 \times 10^{-3} \text{ K}^{-1}$ |
| π | | 3.14159 |
| ε_0 | permittivity of vacuum | $8.8542 \times 10^{-12} \text{ As/Vm}$ |

0.3 List of Variables

| Symbol | Description | Unit |
|--------------------------|------------------------------|----------------------------|
| A | area | m^2 |
| b | width | m |
| B | analogous to a capacitance | F |
| C | capacitance | F |
| D | diffusion coefficient | $\text{m}^2 \text{s}^{-1}$ |
| f | frequency | Hz |
| h | height | m |
| I | DC current | A |
| k | variable | — |
| l | length | m |
| L | characteristic length | m |
| n | number | — |
| P | perimeter | m |
| Pe | Péclet number | — |
| R | electrical resistance | Ω |
| Re | Reynolds number | — |
| T | temperature | $^{\circ}\text{C}$ |
| ΔT | temperature difference | K |
| U | DC voltage | V |
| U_{pp} | peak-to-peak voltage | V |
| v | average velocity | m s^{-1} |
| Z | impedance | Ω |
| ε_{r} | relative dielectric constant | — |
| η | dynamic viscosity | $\text{Pa} \cdot \text{s}$ |
| ω | angular frequency | rad s^{-1} |

| Symbol | Description | Unit |
|--------|--------------|--------------------|
| ρ | mass density | kg m^{-1} |

