Rapid PCR for Integration in Sample-to-answer Analysis Platforms

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Introduction

Polymerase chain reaction (PCR) nowadays constitutes an important and commonly applied method for a plenitude of diagnostics such **as medical diagnostics of infectious diseases**. Compared to conventional approaches such as Gram staining and cell/bacteria culturing, molecular tests are often not only **faster** but also **yield rather specific information,** e.g. on the type of pathogenic agents present. Results from molecular tests thereby render specific and **highly efficient therapy feasible,** in particular, when implemented in systems providing results directly at **the point of care** (POC). Here, a rather elegant solution to integrate a fast PCR in POC systems is presented, based on the **moving plug concept.**
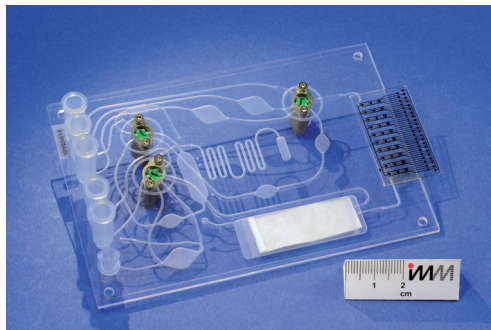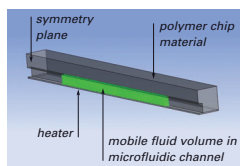

Microfluidic cartridge for HLA typing POC application (EC project CD-Medics) [1].
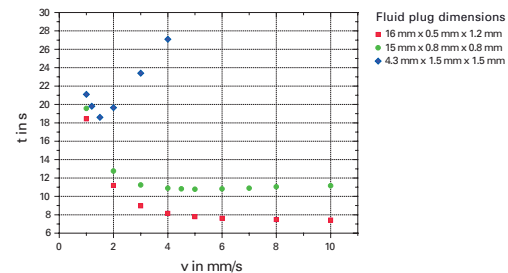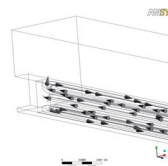
By employing simulation methods such as CFD, optimum heat transfer conditions were identified. Based on these findings a chip layout for fast and robust PCR was devised that runs **30 PCR cycles in 6 minutes.** Most prominently, performance verifications were provided by testing of real samples containing **genomic DNA** both, from purified nucleic acids and not pre-treated **whole blood.** Employing simulation methods and analysing experimental results ended-up in a fast and robust PCR set-up including appreciation of key processes. Notably, the module has the potential of integration to complex sample-to-answer platforms.

System Design and Results

CFD **simulation** (ANSYS CFX) using a moving mesh as fluid plug inside a mesh representing the polymer chip surrounding the channel.
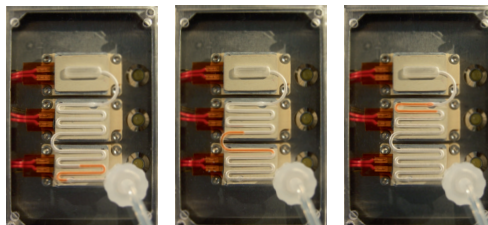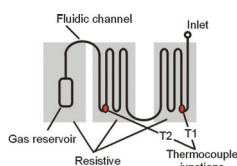

Characteristic **time scales for different transport phenomena.** Diffusion time scales are calculated for half of the channel height:

$$t = x^2 /(2\,D)$$ *t: time, x= 1/2 channel height, D: diffusion coefficient.*

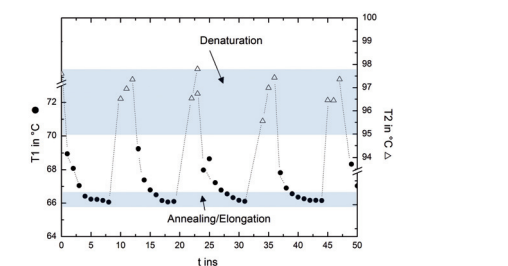| Plug velocity v in mm/s	Internal rotation t in s			Diff. mass transport t in s			Diff. heat transport t in s		
Plug #	1	2	3	1	2	3	1	2	3
10	9.6	6.3	1.7	20	36	111	0.3	0.5	1.9
40	2.4	1.6	0.4	20	36	111	0.3	0.5	1.9
70	1.4	0.9	0.2	20	36	111	0.3	0.5	1.9

Design rationale: The plug is **moved back and forward against a closed reservoir** ("dead end") by air pressure built up by a syringe pump (similar to systems reported in [2, 3]) . Wettability effects such as **corner flow** are **reduced,** because the actuating pressure built up by the pump is significantly higher than the Laplace pressures stemming from surface tension effects. The **dead end reservoir is heated** to **avoid condensation and hence loss of reaction volume.**

The **overall cycling time** is comprised of heating/cooling time and travelling time of the moving plug. Fastest cycling times are found as a compromise between times required when the hold position over a heating zone is reached and velocity of the fluid plug. (high velocity means longer heating/cooling time but shorter travelling time).

Minimum in overall cycling time computed from experimental temperature profiles.

Internal vortices enhance mixing of reagents
⟶ mass transfer is dominated by convection.

Heat transfer dominated by diffusion processes (see **Table** left for estimates of characteristic time scales). Heater is located below the sealing foil of the channel.

Fluid plug (stained by red food colour) **moving from one temperature zone to the next.** (Experiments carried out on a stand-alone PCR device.)

Results obtained during **optimisation of the PCR protocol in terms of cycling time.** ⟶ best results at plug velocity of 10 mm/s and 7 s annealing/elongation.

Fluid plug dimensions
- 16 mm x 0.5 mm x 1.2 mm
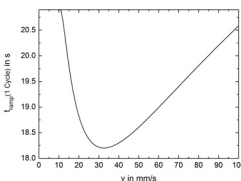- 15 mm x 0.8 mm x 0.8 mm
- 4.3 mm x 1.5 mm x 1.5 mm

From simulations the times t when the final temperature is nearly reached are analysed (here: 98 % of T difference).
⟶ **Heat transfer rates are determined by a) shape (diameter, length) and b) velocity of fluid plug.** A diameter in the range of best performance in terms of rapid heat transfer is chosen. Optimum velocity is determined from experiments.
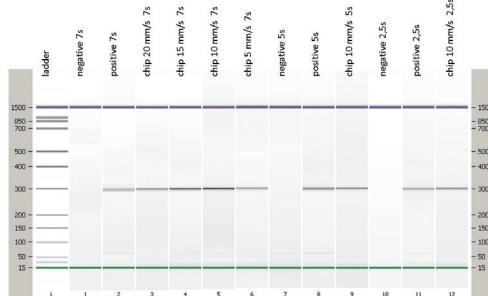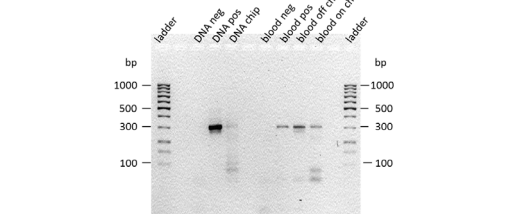

Denaturation
Annealing/Elongation

Typical **temperature profile** of the fluid plug obtained in experiments.

Results from successful PCRs using **purified genomic DNA, centrifuged blood** (blood off chip), **whole blood** (blood on-chip). Positive tests were carried out in a conventional cycler using lysed and centrifuged blood.

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References & Acknowledgement

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