

# **On-Chip Polymerase Chain Reaction with integrated Real-Time Detection**

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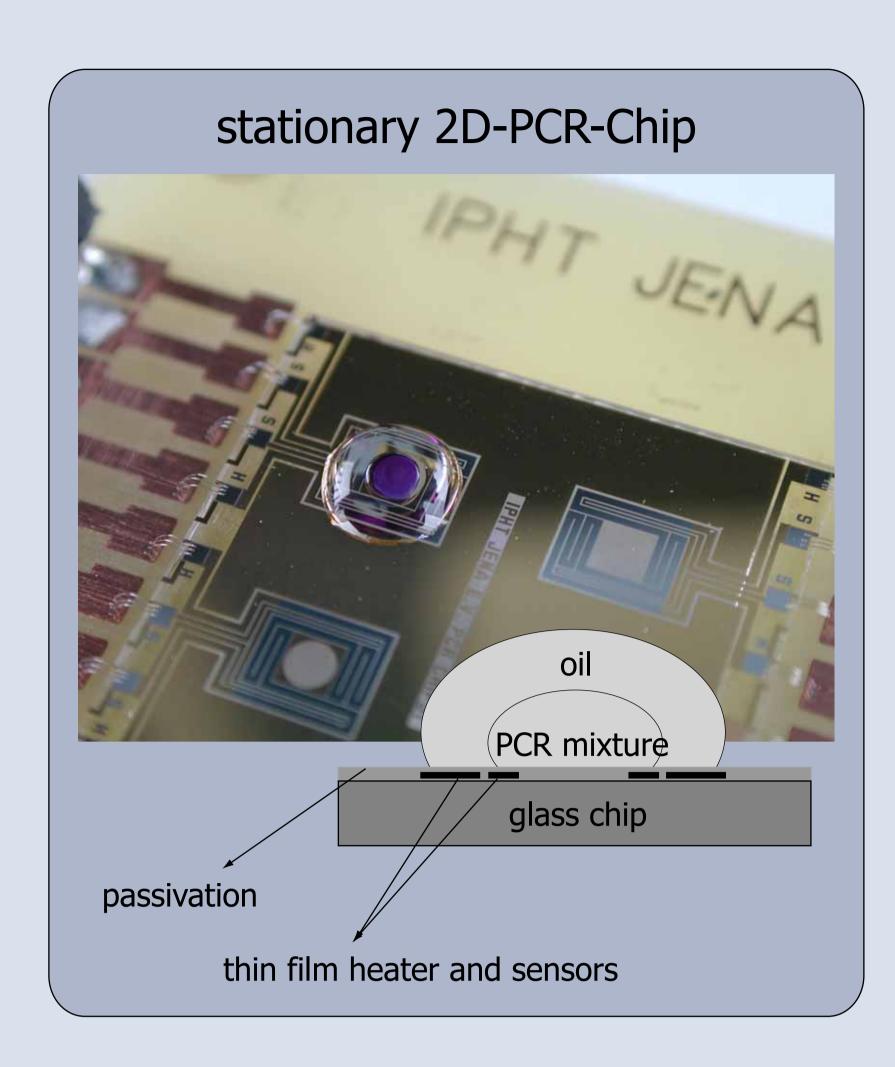
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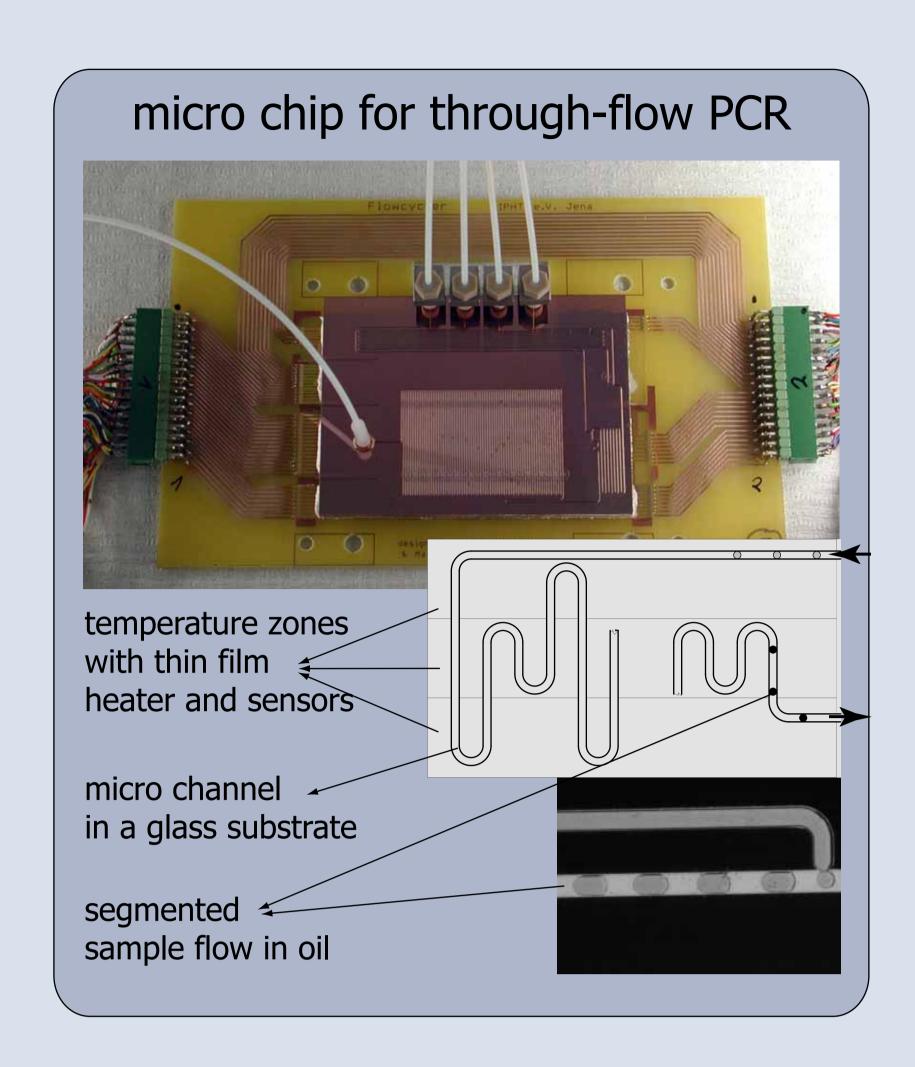
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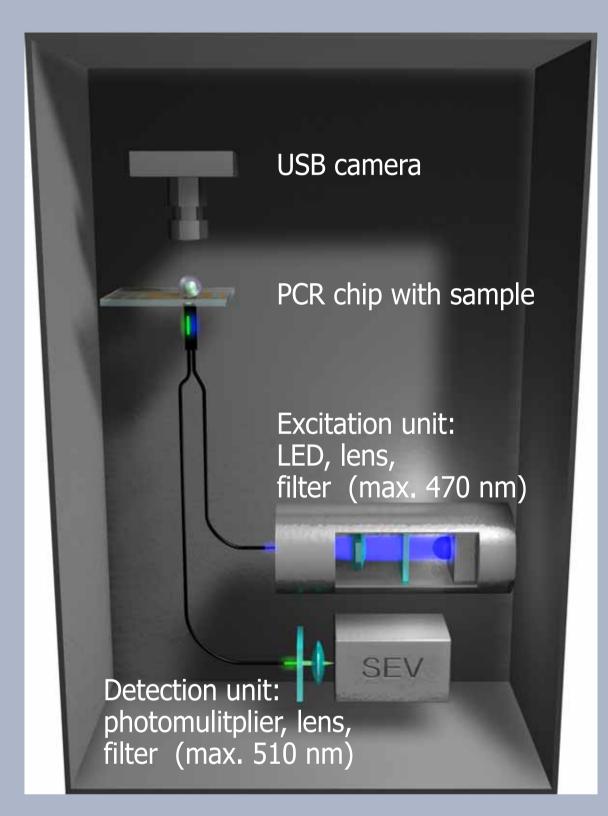
### Micro Chip Thermocyclers for PCR



During the development of PCR micro chips at our institute (IPHT) various strategies were used. Of particular importance were thereby the stationary (fig. left) and throughflow (fig. right) PCR chips. The fabrication of PCR micro chips is based on the micro system technology. Importantly, the micro fabrication technique also provides a possibility for integrating various functional components on the same chip.

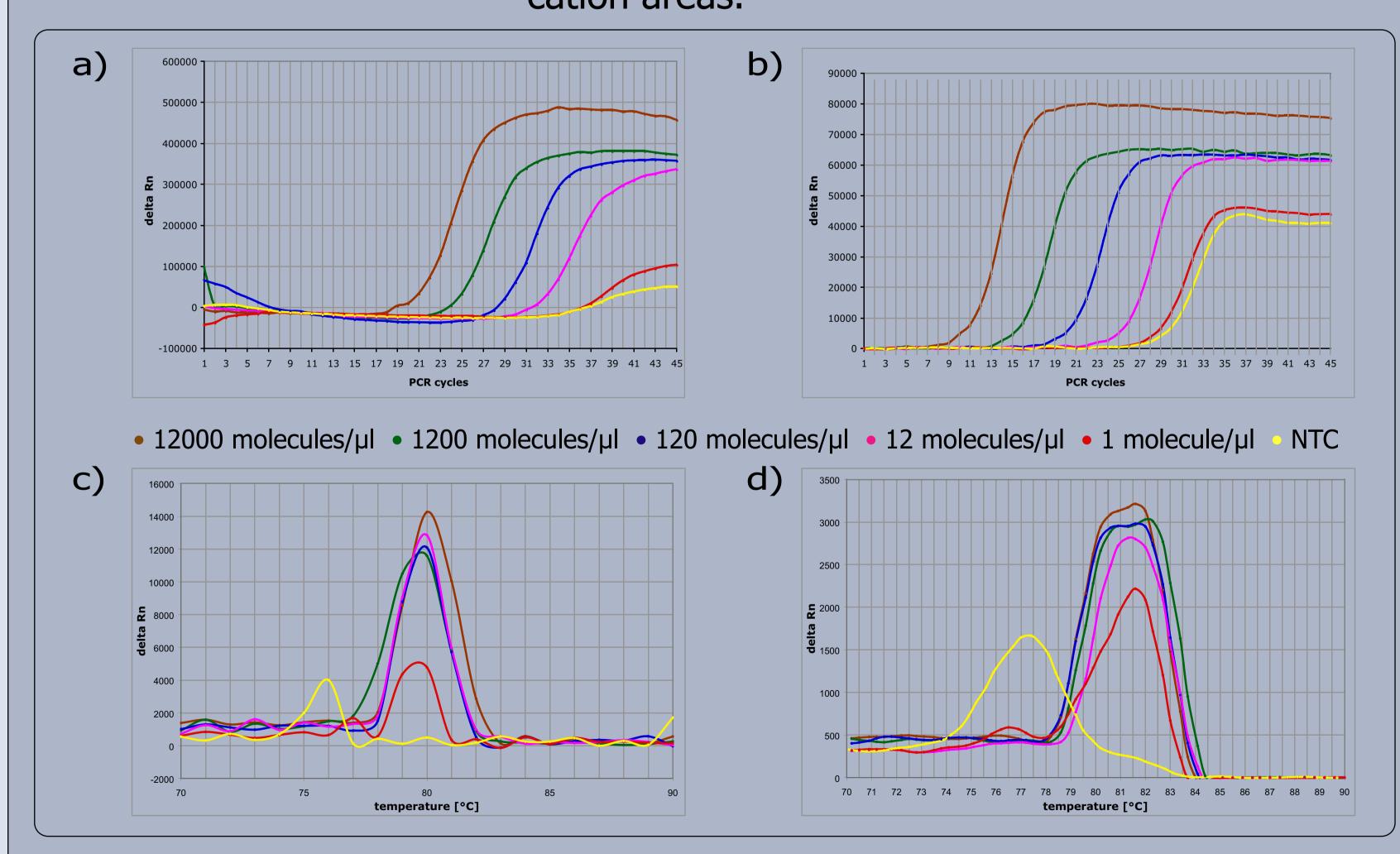


## PCR on stationary 2D-PCR-Chip with integrated Real-Time Detection



On-chip real-time PCR has been proven to provide the same sensitivity and quality of results as conventional systems. But in contrast, due to the high cooling and heating rates, a run of 45 cycles is completed after 23 minutes. At the same time the chip features a very low power consumption and enables on-site analysis.

For real-time detection on the chip (fig. left) we demonstrate results, obtained with Sybr-Green as fluorescence dye. The detection limit was by 1 DNA molecule (fig. a-d) in a sample volume of 1-5  $\mu$ l. In further works we will increase the number of sample/detection sites on the chip for more application areas.



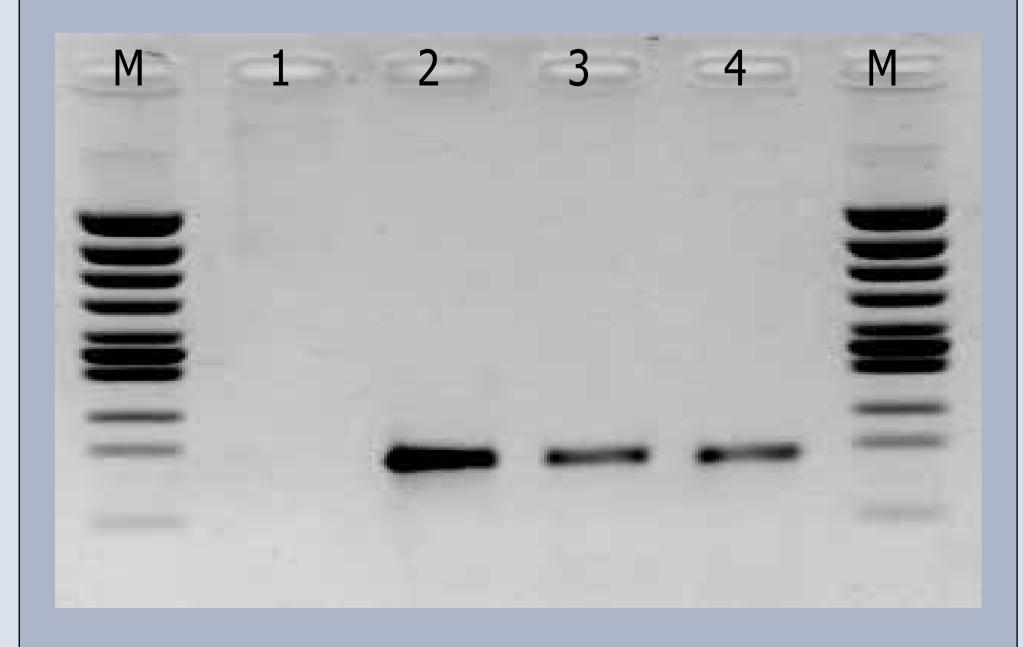
Comparison of the detection at different DNA concentrations between our stationary chip thermocycler and a commercial thermocycler as reference system (ABI 7300, Applied Biosystem).

a) Real-Time detection on stationary Chip; b) Real-Time detection of the commercial cycler; c) Melting curves of the PCR-product on the stationary chip; d) Melting curves of the PCR-product in the commercial cycler

### One-step RT-PCR in a throughflow micro reactor

Our micro chip system for through-flow RT-PCR integrates reverse transcription, hot start activation and cDNA amplification in a single chip module. For the high-throughput analysis of PCR or RT-PCR samples we generate a segmented flow on the fluidic chip (fc). A interesting area of application of the RT-PCR is clinical diagnostics. Therefore, we established a RT-PCR system (fig. below) to detect the oncogen region of Human Papillomavirus (HPV) 16 genome. The vision of that project is the establishment of a system to detect single disseminated tumor cells in the blood of patients with cervical carcinoma.

Our next aim in that project will be the commissioning of a Real-Time detection unit to analyze the PCR/RT-PCR products on the through-flow micro reactor. So it will be possible to reduce the analysis times of the through-flow PCR.



Gel electrophoresis analysis of HPV one-step RT-PCR in through-flow micro reactors with different flow regime in comparison to a commercial thermocycler; M marker, 1 NTC, 2 reference cycler, 3 RT-PCR fc continuous flow, 4 RT-PCR fc segmented flow

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We thank all co-workers of the project partner and of our institute for their contributions to the PCR chips and micro system development. This work was funded by grants of the BMBF (FKZ 16SV1388) and the TMWTA (FKZ B 309-02007).

