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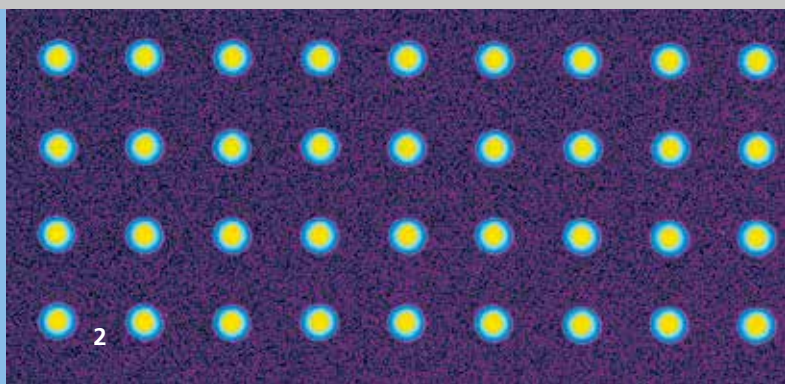

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1 *The analysis system is compact and flexible enough for any doctor's surgey.*

2 *Microarray for hybridization reactions.*

DNA ANALYSIS SYSTEM

COMPACT ANALYZER FOR PCR AND HYBRIDIZATION WITH REAL-TIME DETECTION

Fast and precise DNA detection

In many fields of biotechnology and medical diagnostics, the specific and quantitative detection of DNA is required. This is especially true in the case of pathogens which are responsible for infective diseases. In many samples the DNA concentration is low. Therefor the DNA must be amplified by a polymerase chain reaction (PCR) before detection. For the analysis of viruses associated with specific disease, a set of several DNA sequences should be identified simultaneously. To get quick insitu results the samples should be measured directly at a physician's laboratory or in an outpatient department of a hospital. Therefore a highly automated, compact and cost-efficient system with low time-to-result is developed that allows detection of DNA sequences of relevant virus types in parallel.

Reagent Processing System

The automated system basically consists of a biochip reader and a thermal unit for temperature control and PCR cycling. The software provides a fully automated process control. It can be easily adapted to different applications. A disposable cartridge combining an optical and a fluidic chip is used. The cartridge is realized by low-cost injection moulding processes. The system uses the total internal reflection fluorescence (TIRF) method for real-time monitoring of microarrays. A light beam is coupled into the light guiding slide of the optical chip (Fig.2). All fluorescent molecules which are near to the slide surface are excited by an evanescent field. Heating and cooling is performed by a peltier-element. The temperatures can be reached fast and precisely (Max. temperature rate in chip: 4.5 °C/s).

The camera uses different integration times for measuring a broad range of different

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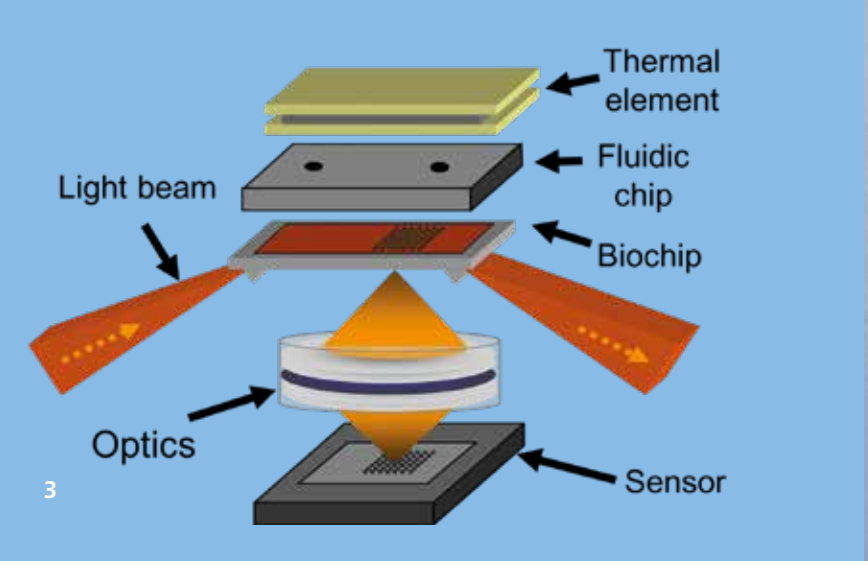

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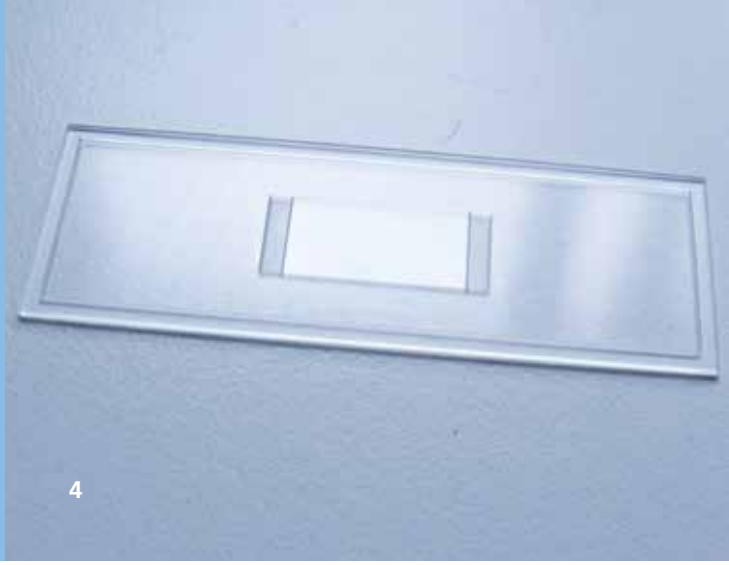

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fluorescence concentrations. The analysis and merging of the images is done automatically by the software. With this method a dynamic range of more than three orders of magnitude can be reached.

Real-time DNA analysis

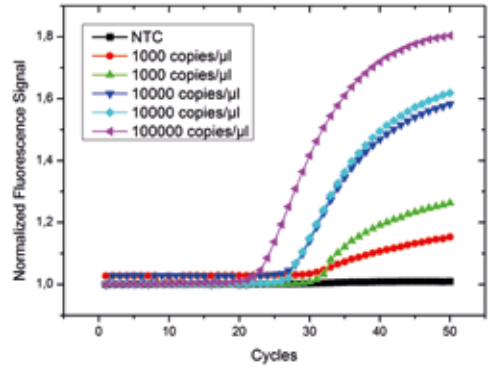
Liquid phase reaction:
A quantitative PCR analysis can be performed within the cartridge. A hydrolysis probe PCR is accomplished in the homogeneous phase in a microfluidic slide. Several channels with a volume < 10 µl enable measurements of different parameters simultaneous. In this manner several samples can be tested in parallel or one sample can be tested for several parameters. A hydrolysis probe PCR enables the real-time detection of the rising fluorescence signal after each cycle. Several PCRs of concentration series of an E. Coli plasmid were performed on the system. Even concentrations lower than 100 copies/µl can be detected. In fig. 4 the resulting graph of a PCR with concentration series of an HPV plasmid is shown. Patient samples of HPV were tested on the system and can be detected.

Solid phase reaction:
The microarray technology allows the simultaneous detection of more than 100 parameters in a small sample. The spots on the surface of a slide contain probes which bind specifically to the targets in the liquid. By TIRF technique the hybridization reactions can be detected in real-time. After hybridization a melting curve analysis can be performed directly for detection of unspecific reactions. First experiments with melting curves and hybridization reactions have been carried out. Fig. 6 shows a hybridization of two different oligonucleotide strands.

Solid-phase real-time PCR

Current developments aim at the combination of PCR and solid phase detection in real-time. As explained above a PCR is accomplished in the homogeneous phase in a microfluidic slide. Real-time detection of the PCR product should be carried out during the PCR process on the slide via a microarray. After the PCR a melting curve analysis can be accomplished for detection of unspecific reactions. This on-chip real-time PCR is a method for quantitative detect of several parameters simultaneously in a short time.

3 ***Set-up with total internal reflection fluorescence (TIRF) method.***
4 ***Polymer slide with integrated microprisms.***
5 ***Real-Time PCR of an HPV plasmid (values normalized to minimum of each curve).***
6 ***Hybridization of a 50 nM solution of two oligonucleotide strands.***



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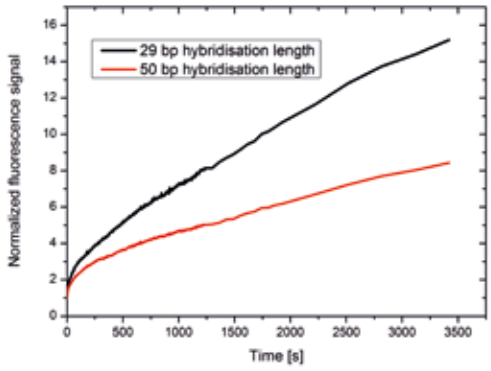

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