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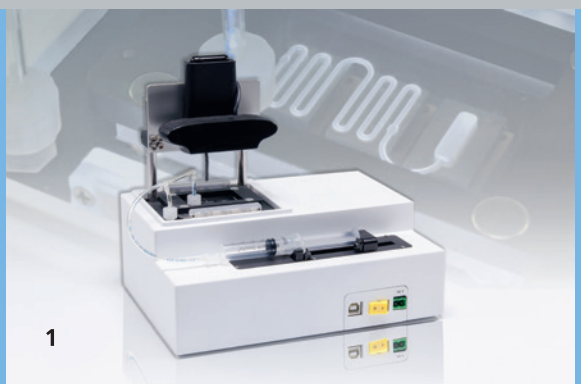

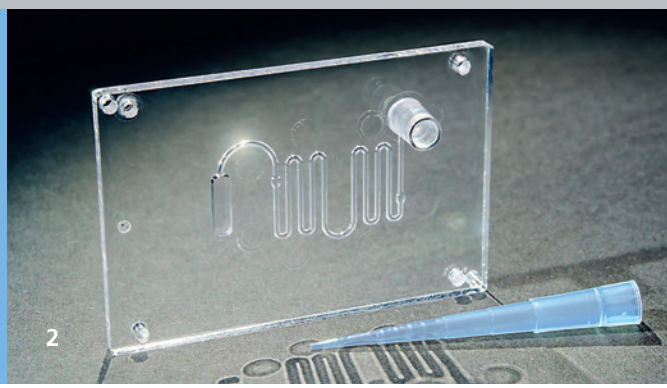

1 *Fully autonomously working moving plug real-time PCR module.*
2 *Disposable polycarbonate PCR chip.*

MOVING PLUG PCR DEVICE – ULTRA-FAST NUCLEIC ACID AMPLIFICATION

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Introduction

Polymerase chain reaction (PCR) nowadays is an important and commonly used technique for a plentitude of diagnostic applications such as medical diagnostics of infectious diseases. Compared to conventional approaches molecular analysis techniques are often faster, more sensitive and have a higher specificity. The result of a PCR test thereby enables a highly efficient and prompt therapy.
Fraunhofer IMM has developed a smart and robust fully autonomously working real-time PCR module based on the moving liquid plug concept. This module enables an ultra-fast PCR capable of running 30 cycles with real-time fluorescence detection in just 6 minutes with the potential of integration into complex sample-to-answer platforms.

Instrument setup

The module consists of an injection molded disposable polycarbonate chip with an adjustable reaction volume that can range from 10 to 25 µl. It has an easy to handle inlet to load the PCR solution. The meandering fluidic channel with a closed air reservoir at the distant end is arranged above two or optional three individually controlled heating zones. During operation, these zones are constantly heated to the required processing temperatures of the PCR, and the PCR solution plug is moved back and forward with a syringe pump pushing against a dead end (Fig. 4). A conventional USB camera allows detection of the plug position inside the chip. In addition, the camera is capable of real-time measurement of fluorescence dyes or probes in the plug. Excitation is enabled by high power LEDs combined with filters.

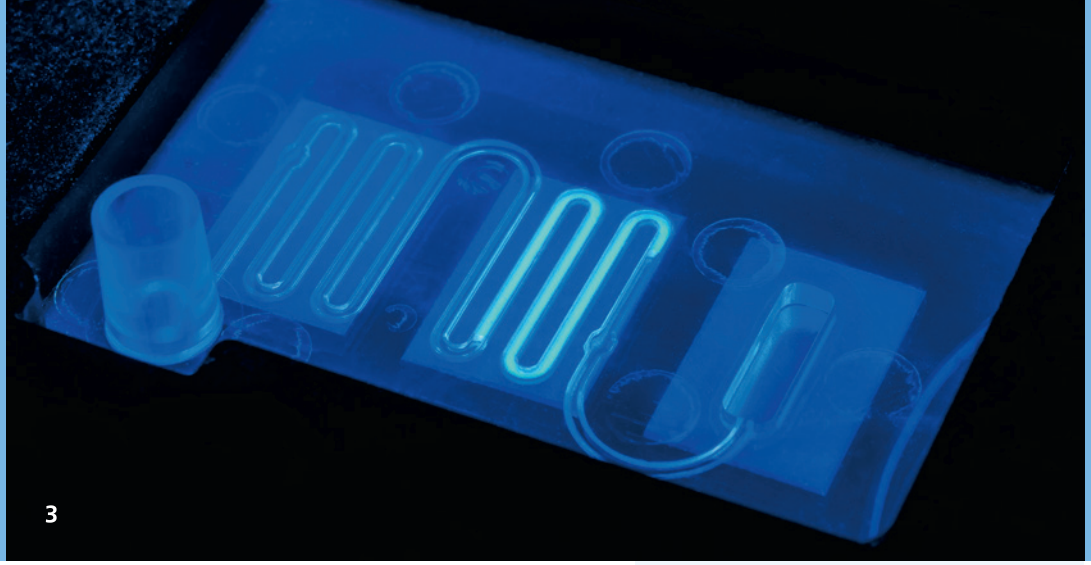

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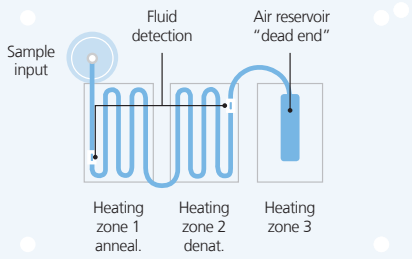
Fig. 4:
Functional schematic of qPCR chip



Competences

Due to the locally fixed heating zones, this new concept omits the cyclic heating and cooling process used in common PCR machines and, hence, leads to an exceptionaly fast temperature change in the PCR solution plug. The periodical movement of the PCR solution causes internal vortices, which enhances an efficient reagent mixing and supports a homogeneous temperature distribution in the plug. The overall cycling time was optimized by simulations (ANSYS CFX) in combination with real sample experiments to reach the best possible compromise between plug speed and heating/cooling time.
With the camera based fluorescence detection the module generates high quality quantitative PCR amplification plots (Fig. 5). In addition, sensitive melting point analysis comparable to data obtained from commercial real-time cyclers can be carried out (Fig. 6). These features provide the user with all information needed to analyze the PCR products.

Experiments showed that the PCR module is not only limited to amplification of purified nucleic acids but is also capable of successfully handling whole blood samples with a direct blood PCR. The PCR solution can be loaded directly into the chip.

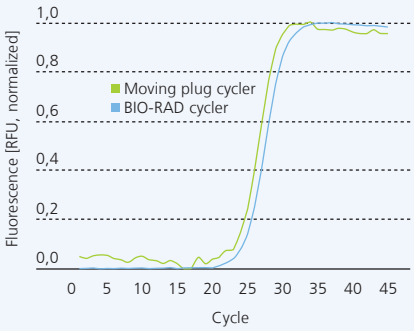
Summary

A compact, ultra-fast, fully autonomously working real-time PCR module is presented. It was achieved by applying latest CFD technology for process optimization enabling best speed and reliable PCR results. 30 PCR cycles in just 6 minutes are possible. Optional fluorescence detection and melting point analyses are integrated in the instrument. It has been shown that the module is capable of handling complex (e.g., human specimen) samples. The reliability of the demonstrator has been proven in several R&D projects. This demonstrator can be run either as fully autonomously working device or as OEM part of a sample-to-answer platform.

References

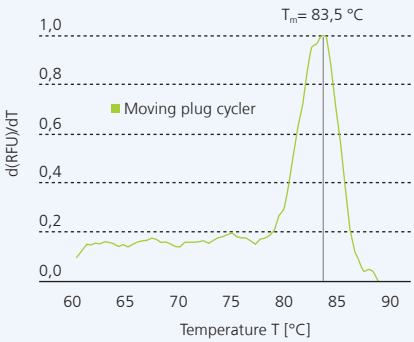
Brunklaus, S.; Hansen-Hagge, T. E.; Erwes, J.; Höth, J.; Jung, M.; Latta, D.; Strobach, X.; Winkler, C.; Ritzi-Lehnert, M.; Drese, K. S.; Electrophoresis, 33(2012)3222, doi: 10.1002/elps.201200259.

Fig. 5: Amplification plots



Comparison between the moving plug qPCR system (SsoAdvanced SYBR Green Supermix) and a commercial cycler (BIO-RAD CFX96) using pEGFP Plasmid as template amplifying a 63 bp amplicon.

Fig. 6: Melting point analysis



Melting point analysis after a successful moving plug real-time PCR of RecF gene (181 bp) from *Baccilus subtilis*.

3 *Fluorescence excitation in a moving plug*