



Microfluidic systems

Moving plug PCR – ultra fast nucleic acid amplification

Quick Facts

- ultra fast real-time PCR within 6 min
- additional reverse transcription and melting curve analysis optional
- low-cost consumables and instrumentation
- compatible with various sample preparation modules
- OEM option for integration in sample-to-answer platforms

Introduction

Polymerase chain reaction (PCR) represents an important and commonly used method for a variety of applications such as molecular infection diagnostics. Compared to conventional approaches, molecular analytical methods are faster, more sensitive and have a higher specificity. However, customary PCR is generally reserved for central laboratories incurring critical report delay due to sample shipment, and considerable costs as well as limited capacities that disqualify laboratory analysis for mass-application.

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 using real-time fluorescence detection and with the potential for integration into complex sample-to-answer platforms.

Amplification of template DNA (plasmid DNA, 30 PCR cycles) showed to be generally feasible within 6 minutes, and detection of pathogens such as SARS-CoV-2 (RNA, RT + 45 PCR cycles) within 15 minutes.

Instrument setup

The module consists of a disposable thermoplastic polymer 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 reaction-mix. The meandering fluidic channel with a distant dead-end air reservoir 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 forth and back with a syringe pump. A conventional USB camera allows for the detection of the plug position as well as real-time measurement of DNA-labelling fluorescent dyes or probes. Excitation is enabled by high power LEDs combined with optical filters.

Microfluidics

The combination of the moving liquid plug concept with heating zones providing constant temperature level areas allows for an exceptionally fast temperature change in the PCR solution plug that is required for a cyclic nucleic acid amplification. In addition, the oscillating 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 a favorable compromise between plug speed and heating/cooling times.

With the camera-based fluorescence detection the module generates high quality real-time PCR amplification plots. In addition, a reverse-transcription step and sensitive melting point analysis comparable to data obtained from commercial real-time cyclers can be performed optionally. These features provide the user with all functional equipment and information needed for PCR-based analysis.

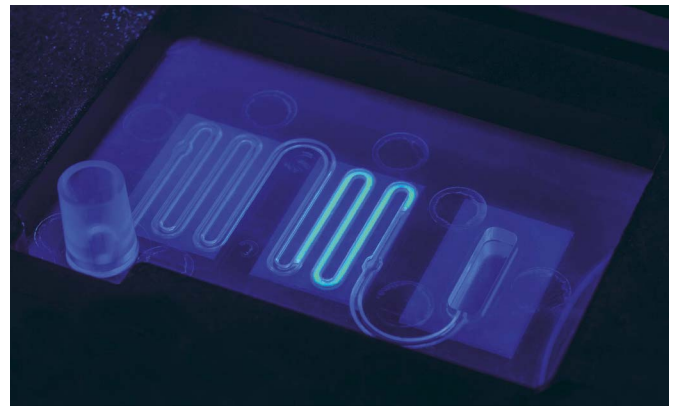
Experiments showed that the PCR module is not only limited to amplification of purified nucleic acids but is also capable of successfully handling whole blood samples with a direct blood PCR. The PCR solution can be loaded directly into the chip.

Division Diagnostics at a glance

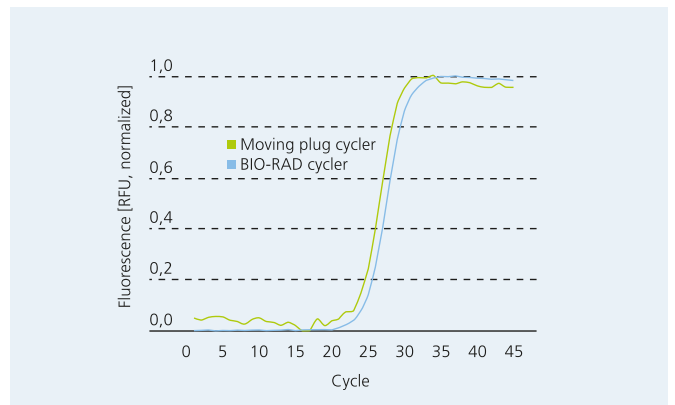
We provide R&D for microfluidic-based analysis systems with applications in life sciences, medical research and diagnostics, food safety or biotechnology.

Our partners benefit from

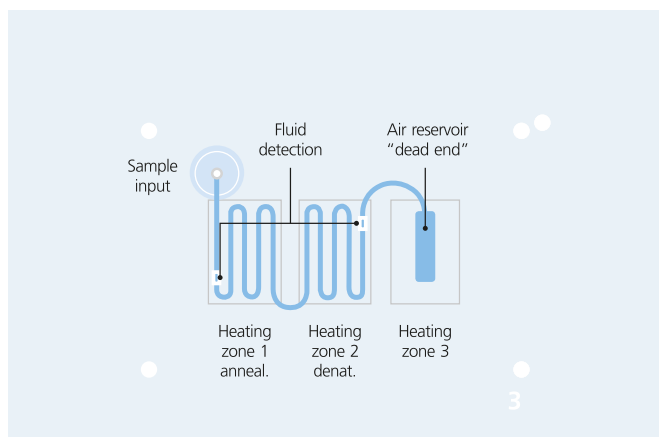
- low cost consumables (disposable)
- minimized reagent consumption for monitoring purposes
- minimal hands-on time or full automation



PCR chip with moving liquid plug.



Comparison between the moving plug PCR system and a commercial cycler (BIO-RAD CFX96) using plasmid DNA as template (pEGFP, 63 bp amplicon).



Functional schematic of PCR chip.

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