Chip Systems for Analysis of Nucleic Acids with Integrated Amplification and Detection

Wolfgang Fritzsche, Mark Kielpinski, Matthias Urban, Thomas Henkel, Sabine Werres, Robert Möller, Stefan Wagner, Marko Riedel, and Sandra Julich

Please note the Erratum at the end of this book.

Abstract Analytical methods for the detection of nucleic acids are suitable for a variety of applications as, for example, the analytical applications in that field are diagnosis of infectious or hereditary diseases, analysis of microbial contaminations, determination of family relations, or identification of genetically modified organisms. In many cases, the available number of DNA or RNA molecules is not sufficient for common detection methods based on hybridization processes. The standard procedure for nucleic acid amplification is polymerase chain reaction (PCR). This thermally controlled, enzymatically catalyzed, and cyclically performed process enables the synthesis of small amounts of nucleic acids.

In this chapter, a microchip thermocycler with integrated heaters and temperature sensors for droplet-based on-chip PCR is described. The chip is coupled with a

W. Fritzsche (✉) • M. Kielpinski • M. Urban • T. Henkel • S. Werres
Nanobiophotonics Department, Institute of Photonic Technology (IPHT), PO Box 100 239, 07702 Jena, Germany
e-mail: fritzsche@ipht-jena.de

R. Möller
Nanobiophotonics Department, Institute of Photonic Technology (IPHT), PO Box 100 239, 07702 Jena, Germany
Present address: Analytik Jena AG, Konrad-Zuse-Str. 1, 07745 Jena, Germany

S. Wagner
Julius-Kühn-Institute (JKI), Messeweg 11/12, 38104 Braunschweig, Germany

M. Riedel
Julius-Kühn-Institute (JKI), Messeweg 11/12, 38104 Braunschweig, Germany
Present address: Landesamt für ländliche Entwicklung, Landwirtschaft und Flurneuordnung, Steinplatz 1, 15806 Zossen, Germany

S. Julich
Nanobiophotonics Department, Institute of Photonic Technology (IPHT), PO Box 100 239, 07702 Jena, Germany
Present address: microfluidic ChipShop GmbH, Stockholmer Str. 20, 07747 Jena, Germany
e-mail: Sandra.Julich@microfluidic-chipshop.com

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temperature controlling unit and a device for measuring the fluorescence intensity. Different amounts of initial DNA down to 2 molecules/μl could be successfully detected. The applied template DNA was obtained from different *Phytophthora* species, a group of phytopathogenic organisms with worldwide significance. Small numbers of cells could also be applied and added directly to the PCR mixture. This was studied successfully with human brain microvascular endothelial (HBME) cells and with spores from *Phytophthora*.

Furthermore, another lab-on-a-chip system is presented that combines on-chip PCR and microarray technology within one chip. The chip consists of a DNA chip with integrated electrode structures and a silicone-based chip with incorporated microchannels and reaction chambers. For temperature management, there are heating elements and temperature sensors connected to the chip. Fluidic transportation is implemented by small peristaltic pumps that are installed to the system. Detection is based on enzymatically catalyzed silver deposition. In case of successful hybridization, the microarray spots turn gray or black, and the electric resistance at the electrode gap will decrease. So the hybridization can be analyzed either by optical-based measurement of the gray scale value or by electrical readout of the electrodes.

Advantages of lab-on-a-chip systems are low necessary amounts of reagents and power supply, short reaction times, and small dimensions of the systems. These make the lab-on-a-chip system transportable and, therefore, well suited for point-of-care applications.

Keywords Lab-on-a-chip system • Microarray • Real-time PCR • Diagnosis • *Phytophthora* • Human brain microvascular endothelial cells

Contents

1 Introduction

Bioanalytical methods for the analysis of nucleic acids, such as polymerase chain reaction (PCR), hybridization techniques, or electrophoresis, are important in diagnostics and research. Due to the identification of specific sequences or sequence variations, there is an enormous potential of applications for nucleic acid-based analysis. Identification of organisms or species, determination of infectious or hereditary diseases, cancer diagnostics, identification of genetic modification, determination of family relations, and even pharmacogenomics for individualized

therapy are examples for bioanalytical applications based on analysis of nucleic acids. Within the technical progress today, a lot of research is done to translate these methods into miniaturized scale [2]. In this context, the concept of lab-on-a-chip technologies is often mentioned [1, 5, 9, 11, 14]. The implementation of bioanalytical processes into microchip scale provides advantages toward conventional applications, like less requirements of material, reagents, energy, and space [18]. Especially, faster performance of temperature-dependent reactions is possible because of less thermal mass. Although there is a great economic interest in saving time and costs, another aim of lab-on-a-chip development is the integration of all necessary laboratory processes for a diagnostic analysis into one system. Ideally these systems are suitable for point-of-care analysis, whereby long transportation distances to specialized laboratories become no longer necessary.

The number of scientific articles about lab-on-a-chip developments and applications is still increasing. Aspects of microfabrication and microfluidics must be considered in developing miniaturized biochips. Biocompatibility of materials plays an important role as well. There are a lot of developments transferring single analytical steps into chip-scale, like amplification of nucleic acids or electrophoresis. For nucleic acid amplification, different types of PCR chips have been constructed [6, 12, 30]. The PCR consists of three steps that are implemented at different temperatures. At first, DNA is denatured at 94°C, and then short single-stranded oligonucleotides that are complementary to the borders of the DNA sequence that has to be amplified anneal to the appropriate areas. Thereby the annealing temperature depends on the length of these primers and their guanine and cytosine content. Normally, the annealing temperature ranges between 50°C and 60°C. In the third step, double-stranded DNA is synthesized by Taq polymerase connecting the nucleoside triphosphates. The three steps are cyclically repeated 25–45 times, whereby ideally during each cycle the number of DNA fragments is doubled.

The reaction mixture is either filled into a chamber device [7, 20, 22] or put as droplet onto the chip surface [8, 23] and heated stationary by adjusting temperatures necessary for DNA amplification. Or, the solution is led through microchannels that are integrated into a chip and guided over different temperature zones [19, 25, 29]. Different chips have also been developed for electrophoresis to analyze nucleic acid fragments according to their length. Thereby often the principle of capillary electrophoresis is applied [4]. To analyze nucleic acids according to their sequence, microarray chips are preferably used [3, 10, 27]. On a microarray, a high amount of different samples represented by capture oligonucleotides with specific nucleic acid sequences is arranged on a small area. By precise positioning, the obtained results can be assigned exactly. Often the amount of nucleic acids that can be extracted from a sample is too low for reliable analysis. For this reason, usually the DNA or selected fragments are amplified by PCR before analysis is performed by hybridization or electrophoresis. Furthermore, the PCR products can be modified simultaneously during the PCR process by application of primers with appropriate labels. The labels can represent, e.g., fluorescent dyes [21] that allow an optical readout of the microarray after hybridization. Because the combination of nucleic acid amplification and analysis is very useful, combined chip systems integrating

both parts have also been developed. Besides a combined PCR and microarray or PCR and electrophoresis, also a fluorescent-based detection of the PCR product during the amplification is possible. The technology of commonly used real-time PCR thermocyclers has also been transferred into chip scale so far.

Another much discussed aspect in molecular biological analysis and especially lab-on-a-chip development is the sample preparation. Although a preceding reverse transcription process can be used if RNA has to be analyzed and also thermally lysed cells were directly added to the PCR mixture, nucleic acid extraction cannot be omitted in all cases. While cellular material like blood is easy to disrupt, others have thick cell membranes or cell walls that necessitate chemical disruption. In that case, a purification of the extracted nucleic acids is essential because the chemicals that are applied for this purpose inhibit polymerase activity during PCR process. Additionally, to lyse cells, nucleic acid extraction and purification have also been implemented on chip systems. While there are a few solutions only for sample preparation [16], combined versions with integrated analysis were also described [13, 15, 24]. In 1990, Manz defined the concept of a Micro Total Analysis System (μTAS) [17], which describes lab-on-a-chip systems that integrate entire processes that are necessary for the analysis of chemical samples in an automated implementation. This concept is absolutely transferrable on biological analysis. Still today the μTAS is the aim of many researching activities, because only in that case the complete laboratory is transferred onto a chip system.

2 Presentation of Two Developed Chip Systems for Analysis of Nucleic Acids

As examples for lab-on-a-chip systems applicable for analysis of nucleic acids, two systems are described in the following. Thereby application fields are demonstrated and appropriate results are shown. As template, different material was applied such as purified DNA or even cells. Also, the technical realization is illustrated including the laboratory setup and the applied chips. In both systems, PCR for amplification of small amounts of nucleic acids or their labeling is implemented. To verify successful amplification and confirm the results of the real-time detection, agarose gel electrophoresis was always carried out. PCR products according to the emerged bands were visualized by ethidiumbromide staining.

2.1 Droplet-Based on-Chip Real-Time PCR

Real-Time PCR offers fluorescence-based detection of PCR products already during the amplification process, thereby saving time as long-lasting analysis procedures of the amplified products subsequent to the PCR process, e.g., by gel electrophoresis, are unnecessary. In the experiments, SYBR green [28] was applied that intercalates into double-stranded DNA and thereby offers especially

quantitative analysis by increasing fluorescence intensity according to the amount of amplified DNA. But the application of SYBR green also offers semi-qualitative analysis of PCR products by implementation of a melting curve analysis [26]. For this purpose, the solution is heated up constantly from 50 to 95°C. Thereby a sudden decrease in the fluorescence intensity can be observed that indicates the specific melting temperature of the DNA fragment. The melting temperature depends on the length of the fragments and their guanine and cytosine content.

2.1.1 Presentation of a Chip System for Real-Time PCR

Laboratory Setup

The laboratory setup for the application of the droplet-based on-chip real-time PCR consists of a box impervious to light including equipment to fasten the chip as well as optical fibers, photomultiplier, and light source (Fig. 1b). For temperature management and data processing, a microcontroller is attached. For parameter setting as well as data analysis, a computer with appropriate software is also connected (Fig. 1a).

Chips

The PCR chip includes microstructured heaters and temperature sensors. These are represented on the chip surface. These thin-film platinum structures are fabricated by photolithographic technologies inside a clean room. In the middle of each structure, there is an optical transparent window that enables fluorescence detection using the combined optical fiber installed underneath the chip for excitation and detection. Each chip contains four of these PCR structures. The chips are attached to circuit boards with arranged electrical connections (Fig. 2a). Via ultrasonic bonding they are connected to the circuit board and at least the temperature control unit.

Because fabrication of the PCR chips is quite expensive and each position can only be used once to avoid cross-contamination, a disposable sample carrier for application on the PCR chip was developed. This sample carrier is a Teflon-coated glass chip with ring structures of untreated glass (Fig. 2b). By these hydrophobic and hydrophilic ring structures, round-shaped droplet consisting of 10 µl mineral oil and 3 µl PCR mixture can be applied on the chip surface (Fig. 2c). Mineral oil is used for prevention against evaporation especially during the denaturation steps.

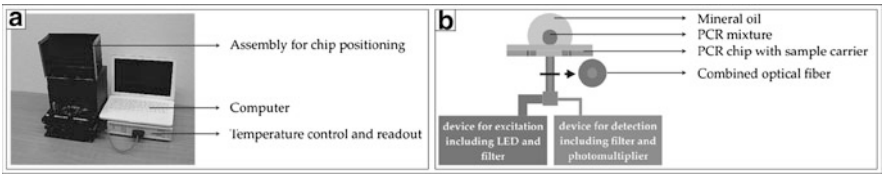

Fig. 1 Laboratory setup (**a**) and detailed scheme of the construction for fluorescence detection during the amplification process occurring inside a droplet arranged on the chip surface (**b**)

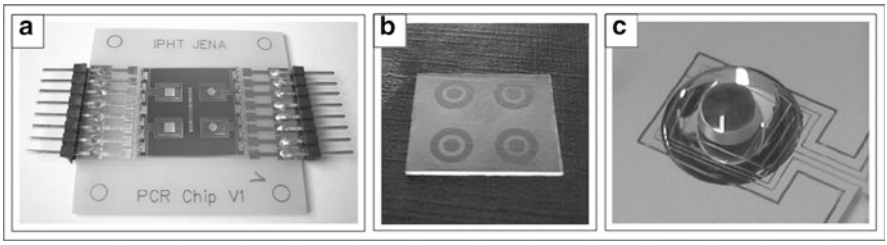

Fig. 2 Entire PCR chip (**a**), Teflon-coated sample carrier with hydrophilic ring structures (**b**), and droplet consisting of clear mineral oil and colored PCR mixture arranged on a heating structure of the PCR chip

2.1.2 Application of Purified Nucleic Acids

Normally DNA serves as template for PCR applications. But RNA or even cells can also be applied, although modification of the protocol is necessary—an additional lysis or reverse transcription step. In the following, results of on-chip real-time PCR are shown whereby extracted and purified DNA was added as initial sample.

Dilution Series

For demonstration of the operability of the PCR chip, a dilution series was applied using initial concentrations between 200,000 and 2 molecules/µl in a total volume of 3 µl of PCR mixture per experiment. All reactions were performed both on the chip and in a conventional thermocycler keeping all parameters identical. The results of the agarose gel electrophoresis (Fig. 3) as well as real-time detection (Fig. 4) demonstrate that PCR on the chip works well and similar to conventional technology. The detection limit with a total number of only six molecules could be achieved in both cases.

Also real-time detection succeeds like expected. With lower initial DNA concentrations, the curve raised up later and the distances between the particular curves were almost consistent. For generation of the standard curves, a threshold value of the fluorescence intensity was defined for measuring the c_t values (crossing points of the curves with a threshold value of the fluorescence intensity). The calculated efficiency for the amplification of the selected DNA fragment from *Phytophthora* using the applied protocol on the PCR chip is 99% and for the conventional cycler 77%.

Analysis of RNA

In case of the detection of a fragment of the coding sequence for the ribosomal protein L13A from HBME cells, either extracted DNA or RNA was applied (Fig. 5a). In case of RNA immediately before PCR, a reverse transcription step

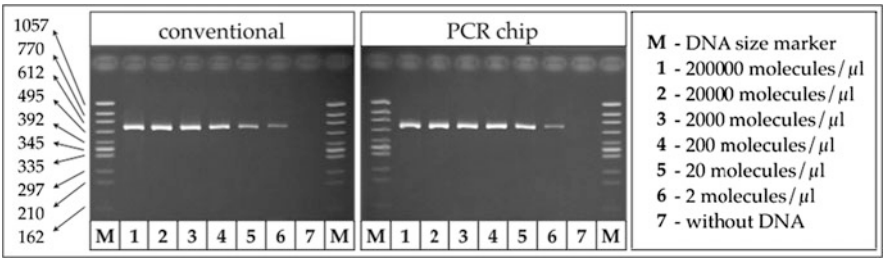

Fig. 3 Results of an agarose gel electrophoresis following PCR on chip and with a conventional thermocycler applying different initial DNA concentrations in a total volume of 3 µl reaction solution

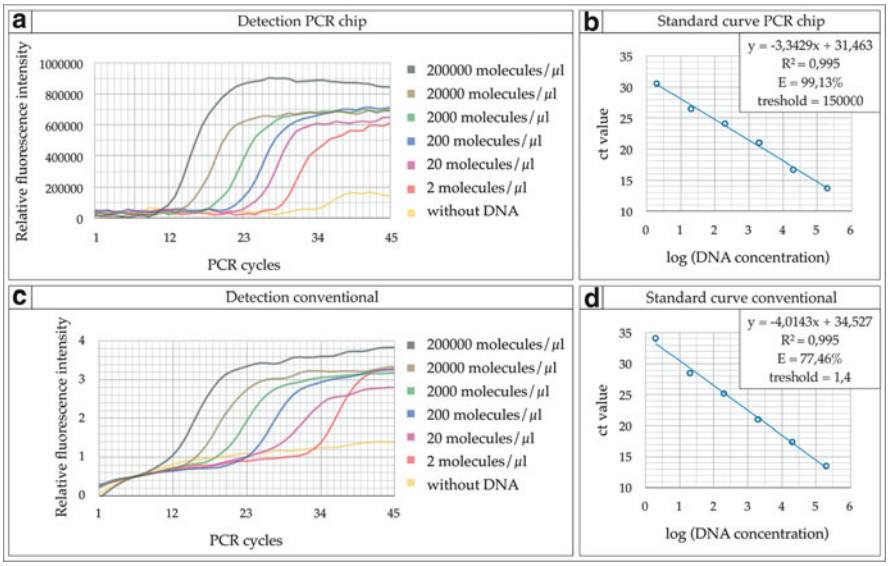

Fig. 4 Results of real-time detection (**a**, **c**) as well as melting curve analysis (**b**, **d**) during amplification of different initial DNA concentrations on the PCR chip (**a**, **b**) and with a conventional thermocycler (**c**, **d**)

was carried out. Therefore in the reaction mixture, not only polymerase but also reverse transcriptase was added.

Multiplex PCR

Multiplex PCR is a beneficial tool for the analysis of several nucleic acid sequences within one tube or droplet. Thereby saving time and material, especially cost-intensive enzymes like polymerase, is possible. Nevertheless, amplification of several DNA fragments simultaneously in one single droplet is a special challenge

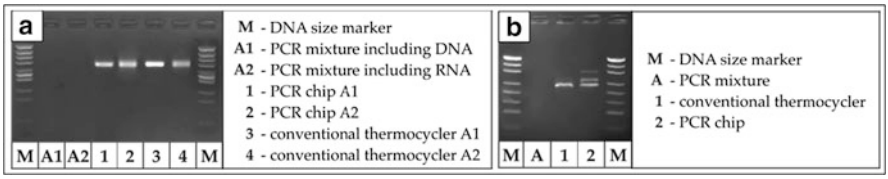

Fig. 5 Results of an agarose gel electrophoresis following PCR on chip and with a conventional thermocycler, on the one hand, applying DNA and RNA from HBME cells amplifying a gene fragment encoding for RPL13A (**a**) and, on the other hand, performing a triplex PCR of gene fragments encoding for RPL13A, gremlin, and vimentin (**b**)

because annealing temperatures of the different primer pairs may only differ marginally. Also inhibition or interference of the PCR products among each other can occur. In some cases, necessary reaction times for multiplex PCR are longer compared to that for amplification of the single products. High variations in the content of guanine and cytosine of the several PCR products can also induce failure of the amplification via multiplex PCR. The agarose gel shown in Fig. 5b demonstrates results of multiplex experiments for amplification of three DNA fragments from purified genomic DNA from HBME cells. An exemplary example for both conventional thermocycler and PCR chip is shown. The selected fragments include gene sections from the coding sequences of the ribosomal protein L13A, as well as the proteins gremlin and vimentin. The experimental results show that in case of the PCR chip, bands for all three products are visible after ethidium bromide staining, although not with homogenous intensity. Using a conventional thermocycler, only the shorter fragments like RPL13A and with a weak band like the gremlin fragment are visible. Therefore, it could be shown that for such particular application, on-Chip PCR is more suitable.

2.1.3 Application of Cells

In modern diagnostics, analysis of cells or even single cells is often required. Therefore the use of cellular material for PCR avoiding time-consuming extraction of nucleic acids is also of great interest today. The identification and discrimination of several *Phytophthora* species are of great interest because some of those parasitic fungus-like species cause plant losses worldwide. Sudden oak death induced by *Phytophthora ramorum* and potato blight induced by *Phytophthora infestans* are famous plant diseases caused by this phytopathogen. Amplification of DNA fragments directly from spores has been successful only by thermal lysis. Therefore, the spores were transferred to the chip surface or into a reaction tube, allowed to dry, frozen at −80°C, and heated at 75°C and 95°C immediately before adding PCR mixture. PCR was performed using the established protocol applied also with DNA as the source material. The success of the experiment was surprising because spores are reproductive structures that are adapted for dispersal and survival for extended periods of time in unfavorable conditions. Therefore, the spores are equipped with thick cell

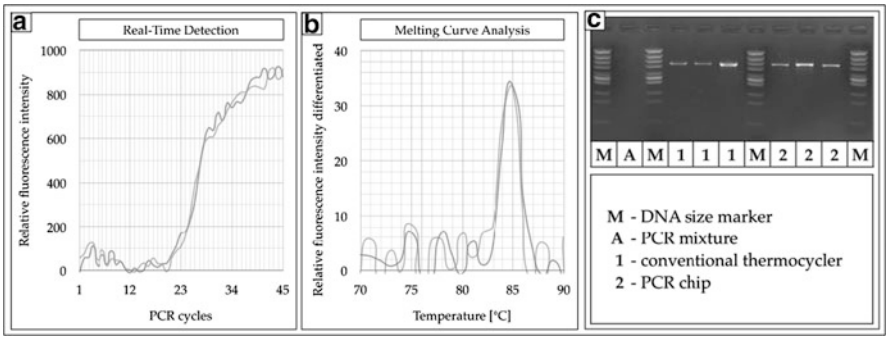

Fig. 6 Results of the on-chip real-time detection (**a**), on-chip melting curve analysis (**b**), and agarose gel electrophoresis of spores direclty added to the PCR mixture

walls. By the visible product bands on the gel shown in Fig. 6c and the detection and melting curves in Fig. 6a, b, it is proven that the spores could be damaged obviously by exposure to cold and heat to make a sufficient amount of DNA accessible for the amplification process. In this case, a number of about 85 spores per experiment were used. For practical application, this number is realistic because spores are produced in high number and thereby could be smeared from the bottom of the leaves for sampling.

In some diagnostic applications, only a few or single cells are available for sampling and analysis. For verification of single cell analysis on the developed lab-on-a-chip system for real-time PCR, single HBME cells were immobilized and thermally lysed appropriate to the *Phytophthora* spores, because in that case, RNA for translation of the L13A protein should be detected by a reverse transcription step to the PCR after adding the reaction mixture to the cells located on the chip or in the tube. Reverse transcription and PCR were performed subsequently, for which reason both enzymes (reverse transcriptase and polymerase) were contained in the mixture. Lysis of animal or human cells is more easy than that of spores because they possess only thin cell membranes that are easy to perforate for extraction of nucleic acids. The results of the single-cell PCR experiments are shown by the agarose gel in Fig. 7c. In both cases, PCR chip and conventional thermocycler-amplified PCR products of the selected fragment with a size of 229 bp are visible, whereas the intensity of the bands according to the on-chip PCR is higher. Also real-time detection and melting curve analysis could be implemented successfully by applying the PCR chip.

2.2 Lab-on-a-Chip System with Integrated PCR and Microarray

Real-time PCR for analysis of nucleic acids is preferably applied for quantitative measurements because the c_t values provide information of the initial content of

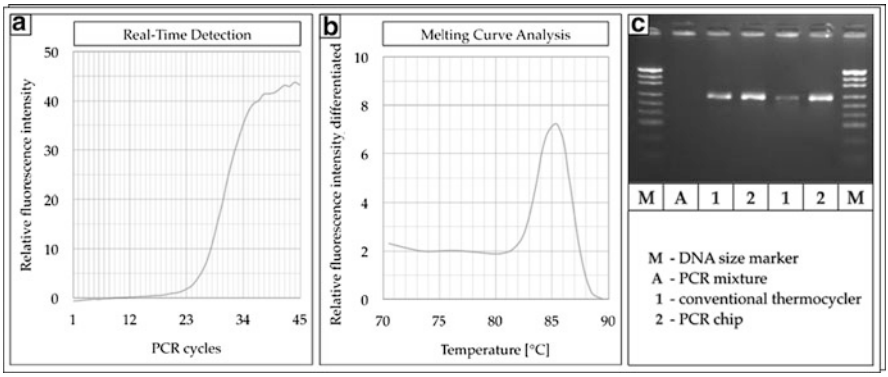

Fig. 7 Results of the on-chip real-time detection (**a**), on-chip melting curve analysis (**b**), and agarose gel electrophoresis of single HBME cells directly added to the PCR mixture

nucleic acids in the PCR mixture. Also a combination with qualitative analysis using fluorescent-labeled probes is possible, but the number of probes that can be analyzed at the same time is restricted. The technical implementation of modern thermocyclers provides a maximum of six different colors for detection in parallel. Also from a biochemical point of view, a high number of different probes and DNA fragments in one sample are not favorable because probability of unrequested hybridization among each other increases. If a sample including nucleic acids has to be analyzed for a high number of sequences or genetic variations, the application of a DNA microarray is preferred. On a microarray, a high number of probes can be immobilized on defined positions within a small area. Therefore hundreds of probes can be placed on about a centimeter. Microarrays are often arranged on small planar chips where special surface modifications for immobilization of the capture probes are required. Under specific conditions and according to the temperature or buffer composition, single mismatches between the sequences of target DNA and capture probe can be determined. The application of a microarray is very useful, e.g., for screening of biological samples of a high number of species according to specific sequence regions. The example that was selected for demonstrating the functionality of that new lab-on-a-chip system with integrated PCR and DNA microarray comprises the differentiation of five different *Phytophthora* species. The presented lab-on-a-chip system includes reaction chambers for amplification and simultaneous labeling of target DNA as well as for hybridization on an applied microarray area. Although the results of that microarray detection can be readout, resistance measurement allows readout already during the hybridization step. For realization of an electrical readout, target DNA is biotin-labeled during the amplification step via appropriate primers, and elementary silver can be deposited after linking of streptavidin-coupled horseradish peroxidase. In case of successful hybridization, the electrode gaps can be closed by deposited silver and an increasing conductivity can be measured.

2.2.1 Presentation of a Chip System for Nucleic Acid Amplification and Detection Via Microarray

A chip system was developed including several technical components for control and measurement. Microarrays offer the analysis of a variety of samples or especially sequence variations within one experiment. For realization of an analytical process using a lab-on-a-chip system, not only the development of the chips itself but also the technical environment including devices for temperature or fluidic management as well as measurement and detection of the obtained results has to be implemented. By developing lab-on-a-chip systems, one's aim should be to construct these technical parts as small and as compact as possible to make the system portable and enable application for point-of-care analysis.

Laboratory Setup

For realization of the identification of several species of a genus using a lab-on-a-chip system with integrated microfluidic structures for amplification and microarray-based detection, a lab-on-a-chip system has been developed and is shown in Fig. 8a. Several technical components are included, such as pumps for fluidic transportation, a device for temperature control, and another one for electrical readout.

The central part of the system is an assembly for chip positioning including Peltier elements for heating and cooling, such as Pt1000 sensors for measurement of the current temperature. The inner construction of this assembly in detail is shown in Fig. 8b. The two heating zones are separated from each other by a gap, whereby thermal crosstalk is minimized. The chip components are placed on the heating plates. Since the microarray is arranged to one side of the chip, this analysis part is connected to one heating zone. The amplification part on the other side of the chip is connected to a second heating zone. The assembly also has a lid that enables to put some pressure on the chip after insertion, whereby tightness of the included microchannels and reaction chambers is assured.

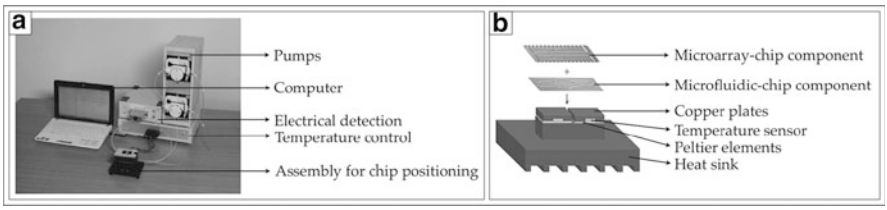

Fig. 8 Laboratory setup (**a**) and detailed scheme of the assembly for chip positioning with integrated components for temperature management (**b**)

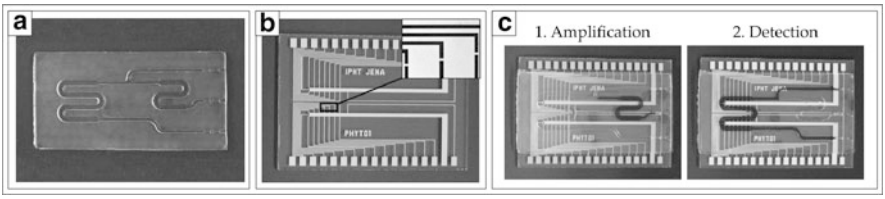

Fig. 9 Microfluidic chip component (**a**), microarray chip component with electrode structures (**b**), and both components after joining with filled reaction chambers for amplification and detection (**c**)

Chips

There are two chip components that are combined to sandwich-like entity. One of these components is a microfluidic chip with integrated reaction chambers and channels for fluid transportation (Fig. 9a). This component is made of polydimethylsiloxane (PDMS), a silicone that is flexible even after the curing process. For fabrication of these chip components, silicone monomers are mixed with a cross-linking agent and the viscous mixture is cast into a molding and hardened overnight. The second component is a microarray chip based on a glass substrate with gold electrode structures on its surface (Fig. 9b). These structures can be fabricated either by microstructuring and photolithography or by application of screen printing which is more inexpensive, especially in large scale.

Because both chip components get in contact with the reaction mixture and the sample, their application as disposables is absolutely necessary whereby cross-contamination is inhibited. Therefore, an inexpensive production of the disposable chip components and appropriate hoses is advisable. After combination of both chip components, the first reaction chamber (S-shaped) will be filled with PCR mixture, and in the second step, the amplified and labeled PCR products will be transferred to the precooled M-shaped microarray zone with simultaneous addition of hybridization buffer (Fig. 9c).

2.2.2 Detection and Differentiations of Several Species of the Genus *Phytophthora*

With the help of the described chip system that is one example for miniaturized nucleic acid analysis, it could be demonstrated that the combination of on-chip PCR and DNA microarray technology is well suitable for detection and discrimination of several species from a biological sample. As one example the differentiation of several *Phytophthora* species could be successfully implemented on that system. Figure 10 shows successful results of the chip-based PCR performed inside the S-shaped reaction chamber of the chip device. It could be demonstrated that the amplification process works similarly and reproducible to those applied in conventional thermocyclers. The selected primer pair not only works well in case of all selected *Phytophthora* species with high phytopathological relevance, but is also

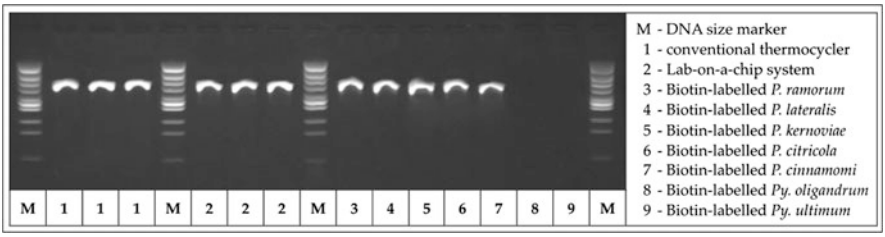

Fig. 10 Results of an agarose gel electrophoresis following PCR on chip and with a conventional thermocycler including verification of reproducibility and specificity of the genus *Phytophthora*

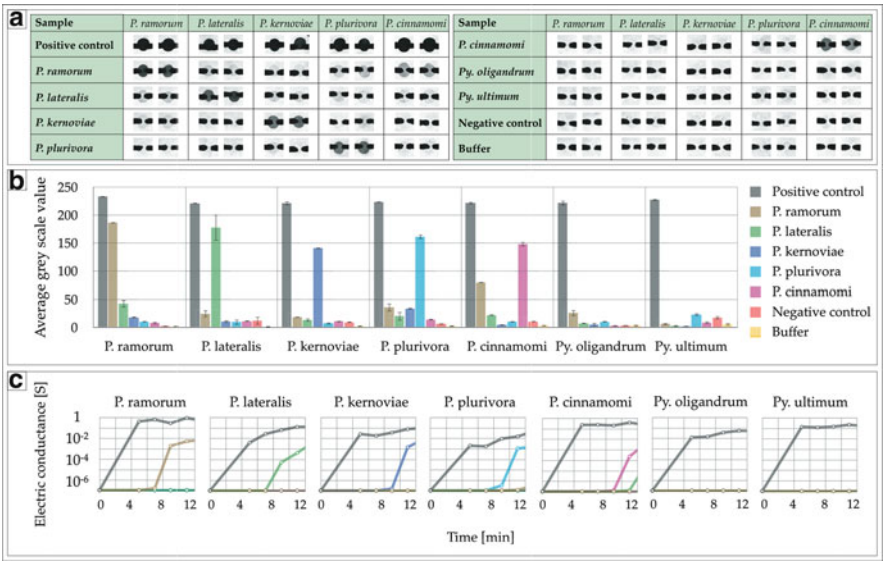

Fig. 11 Optical micrographs from the electrode gaps (**a**), results of corresponding gray scale analysis (**b**), and electrical conductance measurement as a function of time (**c**) after analysis of the DNA from different selected *Phytophthora* and *Pythium* species

selective in case of closely related phytopathogens from the genus *Phytium*. The primer sequences are located on the 5.8 S and 28 S rDNA section of the genomic DNA of *Phytophthora*. These rDNA regions are highly conserved in case of the several *Phytophthora* species, but there are differences between several genera.

The amplified DNA fragment includes a highly variable internal transcribed spacer region that should be applied for discrimination of the several *Phytophthora* species. By means of the light optical micrographs shown in Fig. 11a and the associated results of the gray scale analysis shown in Fig. 11b, it could be demonstrated that all selected *Phytophthora* species are identifiable by optical

detection after the analytical reaction is applied on the chip system. The electrical readout is already permitted during the silver enhancement process performed inside the chip. Therefore, the electrical conductance is measured as a function of time. The results of that electrical detection are shown in Fig. 11c and demonstrate that only in case of the positive control or consistence of template and capture DNA, an increasing conductivity can be measured. The reason for the staggered increase in conductivity from positive control compared to hybridized samples is a higher amount of biotin-labeled probes on the appropriate spots according to the positive control, because the capture molecule consists of direct labeled oligonucleotides. The lower amount of biotin-labeled PCR products is caused by a renaturation effect of the DNA during hybridization, whereby not all capture molecules are coupled with the target DNA. In conclusion, electrical detection is more sensitive and selective because there is almost no background signal detectable because conductivity can only be measured if there is a continuous silver layer on the spot.

3 Summary

According to the obtained results that were achieved by applying the represented lab-on-a-chip systems for analysis of nucleic acids, it could be demonstrated that a combination of nucleic acid amplification and detection is well feasible in miniaturized scale. Both fluorescence-based real-time detection and analysis on electrically readable microarrays can be implemented on chip. For that reason two different lab-on-a-chip systems have been constructed. On-chip real-time PCR is particularly well suited for quantitative analysis because fluorescence intensity is always directly related to the amount of DNA contained in the PCR mixture. Qualitative measurements are also possible, but the number of sequences that can be analyzed in parallel is restricted. For the purpose of analyzing a template according to a high number of sequences, the combination of amplification and simultaneous labeling with a microarray is preferable. Detection of hybridized nucleic acids by enzymatically catalyzed silver deposition is a promising alternative to fluorescence-labeled target DNA because electrical readout can be carried out quite fast and has no need for complicate detection equipment. Also direct application of cellular material such as HBME cells or spores obtained from plants infected by *Phytophthora* was successful, and thereby time-consuming extraction of nucleic acids could be omitted. Presented systems offer fast analysis with low requirements of material, power, and especially initial sample. Complete analysis within a few hours is enabled by using the applied protocol, but there is also potential for time-dependent optimization. If point-of-care application is possible, time-consuming transportation could be minimized.

4 Conclusion and Outlook

On the basis of the developed chip systems, it could be demonstrated that nucleic acid analysis is well convertible in miniaturized scale. Although the development of lab-on-a-chip systems is still advanced and first systems are already commercially available, they did not really find their way in routine diagnostics yet. Further development regarding robustness and automation as well as smaller and faster implementation are required.

Nevertheless, it is not always possible to do nucleic acid analysis of complex biological samples without DNA extraction; processes of cell lysis or nucleic acid purification also have to be adapted in miniaturized scale for implementation of the complete analytical procedure in one single chip system. Because different methods are necessary, according to different analytic or diagnostic questions, there will not be one universal lab-on-a-chip for nucleic acid analysis at all because the technical equipment around the chip would be too extensive to achieve a compact system that is portable and adequate for point-of-care application. Rather several systems for special applications or different analytical questions should be developed.

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