PCR and microarray chip technologies for *Phytophthora* diagnosis

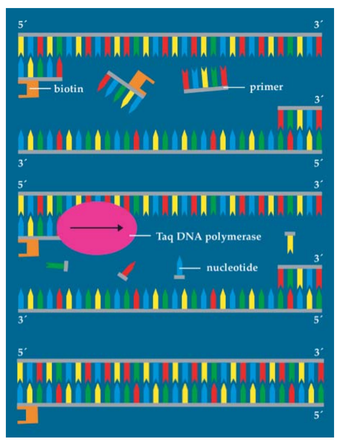
Sandra Julich[1], Mark Kielpinski[1], Matthias Urban[1], Marko Riedel[2], Stefan Wagner[2], Sabine Werres[2], Wolfgang Fritzsche[1], Robert Möller[3], Thomas Henkel[1]

1 | Institute of Photonic Tehcnology, Albert-Einstien-Staße 9, 07747, Jena, Germany
2 | Institute of Plant Protection in Horticulture and Forestry, Federal Research Centre for cultivated Plants, Messewg 11/12, 38104, Braunschweig, Germany
3 | Institute of Physical Chemistry, Friedrich-Schiller-University; Helmholzweg 4, 07743; Jena, Germany

The polymerase chain reaction (PCR) is one example for a standardized method in molecular biology that is well transferable in miniaturized technology. Often the amount of nucleic acids extracted from biological material is not adequate to allow significant detection. The PCR provides amplification of only a few DNA molcules whereby identification of DNA becomes possible. One possibility of DNA detection is hybridization on a microarray. In this case differrent methods for signal readout are applied using fluorescence-based or electrical detection. Our aim is to combine a stationary PCR-chip with an electrical DNA-chip for performance of amplification and detection of nucleic acids on one workflow using a single chip that is inexpensively fabricated of glass and PDMS. Therfore only small amounts of sample material are neccessary and fast performance of the whole reaction is realizable.

PCR-chip module

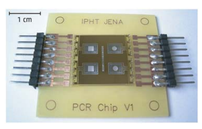
We have developed a stationary PCR-chip with integrated microstructured thin film heaters and temperature sensors on its surface. The reaction mixture is filled into a disposable chip that includes reaction chambers and prevents against cross contamination.



Principle of DNA-amplification with integrated biotin-labeling

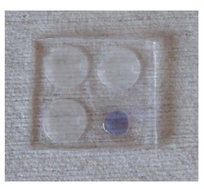
M - DNA size marker
A - PCR mixture
1 - Conventional Thermocycler
2 - Reaction chamber on chip
3 - Droplet on chip

Results of PCR demonstrating funktionality of the PCR-chip modul



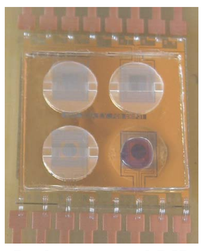
Stationary PCR-chip

+



Reaction Chamber

=



Performance of PCR

Application: Phytophtora detection

Many species of *Phytophthora* are plant pathogens of considerable economic importance. Some of them are regulated as quarantine organisms. To detect latent and non-latent infections presuppose a fast point-of-inspection diagnosis which is species specific.

Disease symptoms caused by *Phytophthora*

P. ramorum/Rhododendron spec.

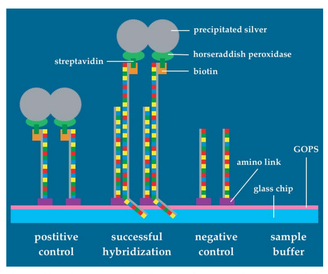
P. ramorum/Umbellularia californica

P. fragariae/Fragaria x ananassa

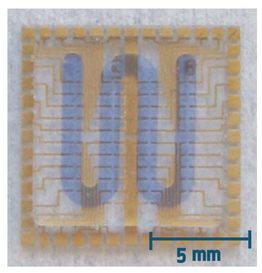
P. porri/Allium porrum

DNA-chip module

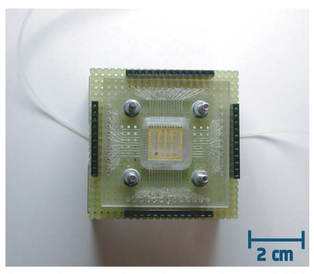
Our DNA microarray-chip with integrated electrode structures allows enzymatic catalyzed silver enhancement and electrical readout of the signal after hybridization. The spot positions are on electrode gaps that are bridged in case of successful hybridization. Advantages of flow trough hybridization are savings in reaction time and reagent volumes as well as enhanced measurement results compared with conventional hybridization chambers.



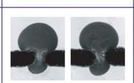
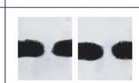
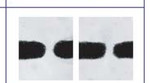
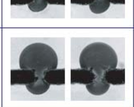
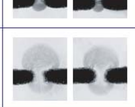
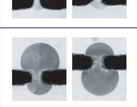
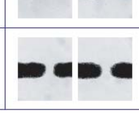
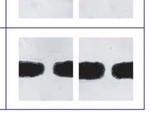
Applied principle of nucleic acid detection



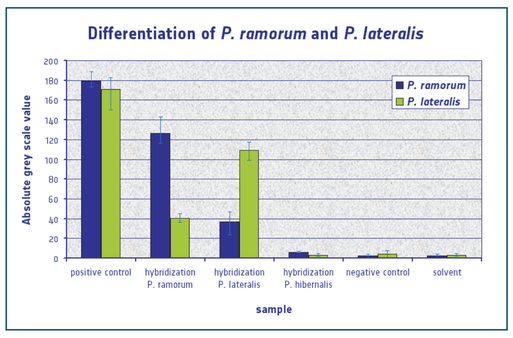
Electrical DNA-chip with fluid channel

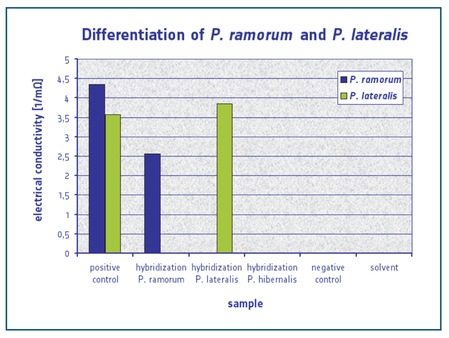


Reaction chamber for DNA hybridization

sample	positive control	hybridization P. ramorum	hybridization P. lateralis	hybridization P. hibernalis	negative control	solvent
P. ramorum						
P. lateralis						

Micrographs after hybridization of PCR products from the species *Phytophthora ramorum* and *Phytophthora lateralis*

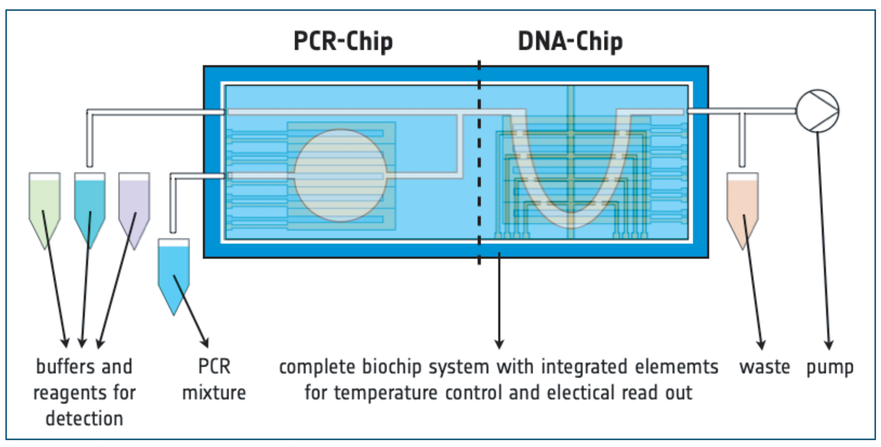




Results of optical (left) and electricel (right) readout (average values of three independent experiments)

Development of a complete chip system

The aim of an actual project is the combination of both PCR and DNA chip technologies to develop a platform for amplification and labelling of DNA and its qualitative detection. This complete system with integrated fluidic and thermal management will be combined with appropriate measurement and control technology. Disposable chip devices and compact dimensions enable point-of-care applications in different diagnostic fields.



Scheme of the biochip system for molecular biological analysis

IPHT – Institute of Photonic Technology
Microfluidics
Phone +49 [0] 3641 206 307
E-Mail thomas.henkel@ipht-jena.de
www.ipht-jena.de

Acknowledgments
We thank all co-workers of the project and of our institute for their contributions to the bio-chip system development. This work was founded by grants of the Federal Agency for Agriculture and Food. (Project "PhytoChip", FKZ 28-1-42.027-06)

Federal Agency for Agriculture and Food