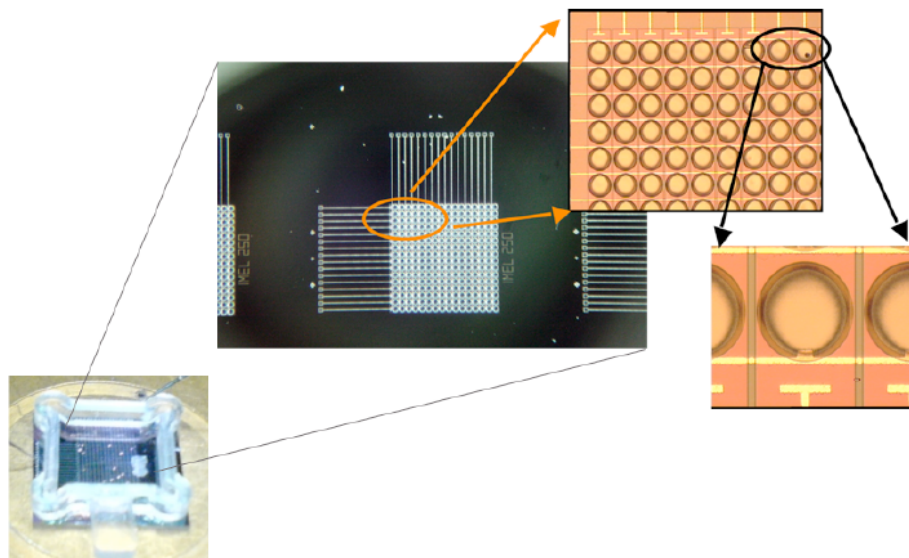


2 MicroDNA

*Integrated polymer-based Micro fluidic
Micro system for DNA extraction,
amplification, and silicon-based
detection*

exploded view of the capacitive biosensor array



INTRODUCTION

- ◆ The Micro2DNA research focused on the development of a Lab-on-Chip
- ◆ The Lab-on-Chip prototyped in the project is integrated in an instrument that performs the signal transduction, interpretation and data analysis.
- ◆ The whole system is interfaced by dedicated software (Data Manager Application) developed to make the operation of the enclosing instrument by general practitioners efficient and straightforward.
- ◆ The project addressed also three different healthcare applications, namely Cystic Fibrosis, β -Thalassaemia and Cardiovascular diseases. For each application optimized assays were developed for optimal performance with the system prototype.

The Consortium



ICOM



TYND



LETI



IMBB



NTE



SENS



SCION



NTUA



OXFD



UCPR



NCSR

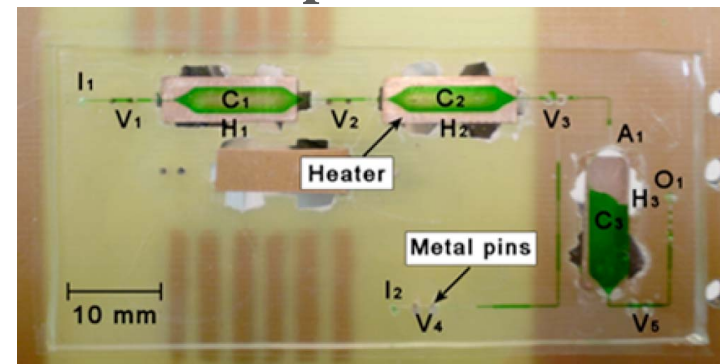


CTRC

Microfluidics TECHNOLOGIES

PDMS based sample preparation

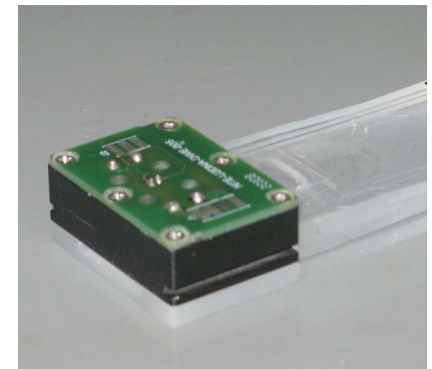
- ◆ The project has successfully fabricated integrated PDMS chips that can enable PCR amplification from whole blood specimens.
- ◆ The moulding techniques used ensure the fabrication of these integrated PDMS chips in a fashion compatible with high volume manufacture.



Microfluidics TECHNOLOGIES (cont.)

Lab-on-Chip Integrated Cartridge

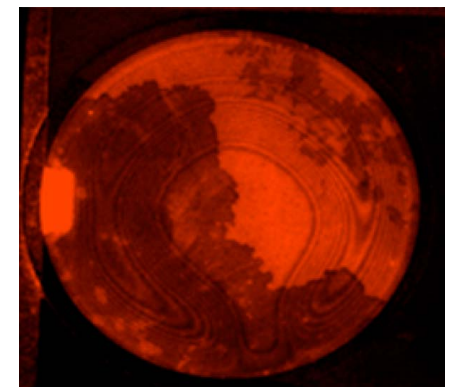
- ◆ The Micro²DNA research focused on the development of a Lab-on-Chip, an integrated micro system for nucleic acid extraction, mixing steps, purification, PCR amplification, hybridization and detection. DNA sample preparation module on PDMS, and Hybridization chamber module on aluminium were integrated on a LoC cartridge that is the disposable device used for the DNA tests.



HYBRIDIZATION DETECTION TECHNOLOGIES

Capacitive Biosensors

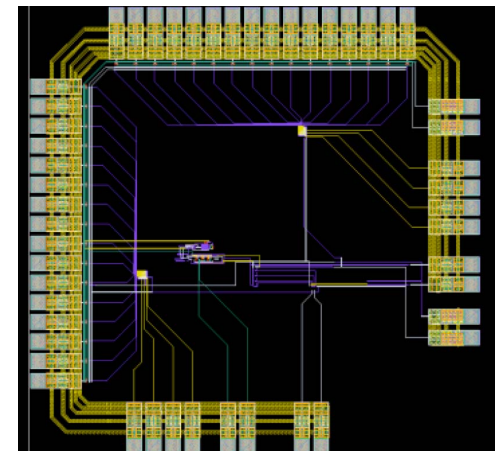
- ◆ Innovative electronic detection was carried out by an array of DNA capacitive sensors implemented on silicon.
- ◆ Dedicated functionalization procedures were introduced and established for immobilization of biotin and oligos on the SiO_2 surfaces of the biosensors.
- ◆ An integrated testbed was developed for the testing of the performance of the sensors. Testing was performed using biotin and β -Thalassaemia samples.



HYBRIDIZATION DETECTION TECHNOLOGIES (Cont.)

Readout ASIC

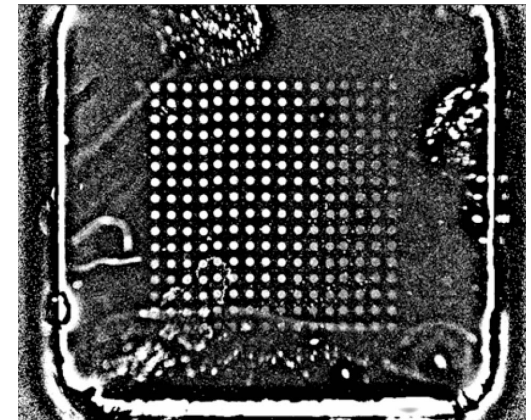
- ◆ A dedicated ASIC for the readout of the capacitive array has been designed, using 0.35um CMOS technology, and fabricated at AustriaMicrosystems.
- ◆ The interface is able to read the whole array of 256 capacitive sensing elements by using a 16 x 16 addressing mode to select the sensor to be measured



HYBRIDIZATION DETECTION TECHNOLOGIES (Cont.)

CCD based Approach

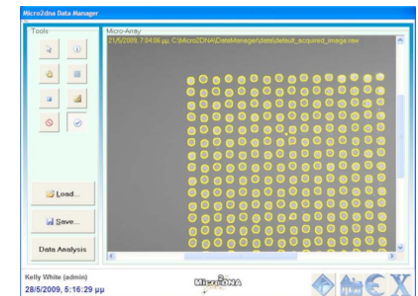
- ◆ A low cost hybridization detection technique was developed and tested. This CCD-based approach finally was adopted for validation in the project. The excitation /detection system involves an evanescent wave (low cost LED) to excite the fluorophores bound on a glass slide and a CCD sensor to collect the emission light. The optical offset is minimized with a matched filter.



Instrumentation TECHNOLOGIES

Instrumentation

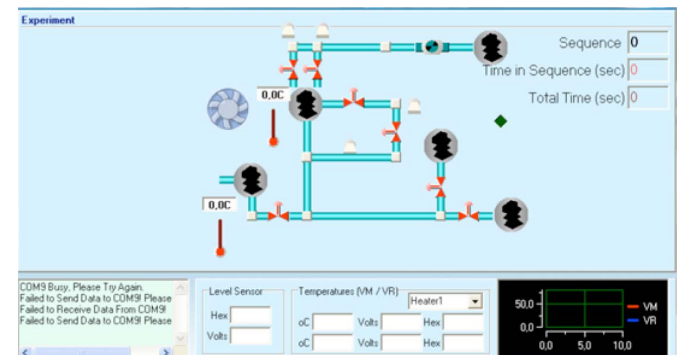
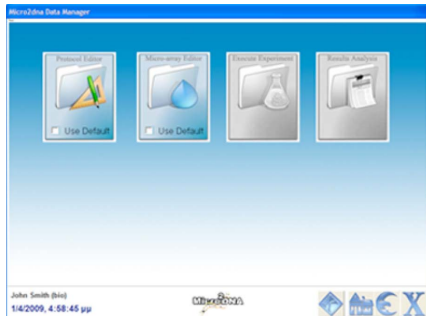
- ◆ An instrument was developed that hosts, monitors and controls the LoC, as well as acquires the image from the attached CCD to analyse the data in order to present the results of the examination to the end user.
- ◆ The instrument features an embedded PC, mixed Analog-Digital circuitry, an additional microprocessor running dedicated firmware for assuring real-time response and all needed mechanical components for the proper operation of the LoC (valves, pumps, temperature sensors, heaters etc.).



Instrumentation TECHNOLOGIES (Cont.)

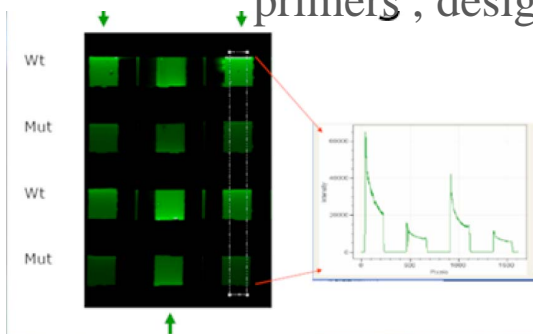
Data Manager Application Software

- ◆ The Micro²DNA instrument is operated by various groups of users through a dedicated software application (Data Manager).
- ◆ It features an environment for describing the flow of the LoC operation and subsequently monitoring its execution.
- ◆ The acquired data from a measurement of an activated LoC are analysed in a fully automated manner and the results are presented to the user in the form of a comprehensive examination report.
- ◆ The Data Manager has been designed so to be possible to be used with other LoC architectures or technologies.



New Assay products for Molecular Diagnostics

- ◆ *Cystic Fibrosis assay* 41 CFT mutations design including 21 international, 6 additional for UK, 13 European, and 1 general. 3 Polymorphisms have been identified that would interfere with AF508 discrimination. PCR primers have been designed to fit into 4 multiplex reactions.
- ◆ *β-Thalassaemia assay* β-globin gene has been divided into 10 amplicons and 28 β-Thalassaemia mutations have been selected and the corresponding isothermal probes have been designed.. PCR primers have been adjusted to fit into 2 multiplex reactions.
- ◆ *Cardiovascular Disease assay* 14 CVD mutations out of 14 amplicons. PCR primers , designed to fit into 3 multiplex reactions



Micro²DNA Achievements

- ◆ PDMS based DNA sample preparation module using whole-blood PCR
- ◆ Hybridization Detection Technologies:
 - ◆ Capacitive biosensors for Hybridization detection,
 - ◆ Readout circuitry on ASIC (capacitance to frequency conversion)
 - ◆ Innovative immobilisation technique of oligos on SiO₂
 - ◆ CCD detection of evanescent-light-illuminated microarrays

Micro²DNA Achievements (Cont.)

- ◆ Lab-on-Chip (LoC) integrated cartridge for sample preparation, hybridization and detection
- ◆ Instrumentation for molecular Diagnostics operating the Micro2DNA LoC
- ◆ Instrumentation Software (Data Manager) with advanced features for:
 - ◆ Controlling (manipulating) the Micro2DNA (or any other) LoC
 - ◆ Monitoring the operation of the Micro2DNA (or any other) LoC
 - ◆ Fully automated analysis of the measurements from the biological reactions on the Micro2DNA (or any other) LoC
 - ◆ Intelligent creation of examination report

Micro²DNA Achievements (Cont.)

- ◆ New Assay products for Molecular Diagnostics Examinations
 - ◆ Cystic Fibrosis assay
 - ◆ β -Thalassaemia assay
 - ◆ Cardiovascular Disease assay

Further future development is required

- ◆ Redesign of the integrated cartridge for industrialization (automate also blood sample insertion in the cartridge)
- ◆ Achieve biologically meaningful operation of the integrated cartridge in the instrument
- ◆ Integrate capacitive sensors in the instrument
- ◆ PUT USERS (Doctors and Test Lab users) to benchmark the instrument fully operational with other technologies by carrying out examinations of the developed assays

μ 2ggen

MICROSYSTEMS FOR MOLECULAR DIAGNOSTICS

Micro²Gen

- ◆ **Micro²Gen (Microsystems-Microfluidics for Genetic tests and Molecular Diagnostics)** is a Microsystems company with products for the molecular diagnostics market and particularly for genetic tests.
- ◆ Micro²Gen aims to develop the All-in-one™ BioChip product, a low cost integrated micro system with modules for nucleic acid extraction, mixing steps, purification, PCR amplification, biochemical reactions and electronic detection in reduced time.

Micro²Gen (Cont.)

- ◆ Portable low cost, point of care system will host the LoC and it will perform the signal transduction, interpretation and data analysis.
- ◆ It will be available to the market for various applications which will be thoroughly clinically validated before entering the market.