SIXTH FRAMEWORK
PROGRAMME

Projet No: IP 026804-2

Project Acronym: DINAMICS

Project title: Diagnostic Nanotech and Microtech Sensors

Instrument: IP

Thematic Priority: NMP

FINAL Activity Report

Period Covered: All (M01 – M48)

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Start Date of Project: 01.04.2007

Duration: 48 Months

Project Coordinator Name: Dr. Christian Mittermayr

Project Coordinator Organisation Name: Lambda GmbH (LAM), AT

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1 Project execution

1.1 Summary Description of Project Objectives

The objective of the DINAMICS project is to develop an integrated and cost-effective warning system for the protection of the civilian population from bioterrorism.

The DINAMICS project aims at developing a sensor technology that will provide required sensitivity and speed of response whilst compatible with integration of DNA microarray technology and nano-biotechnology into a low-cost, high-volume lab-on-a-chip platform.

The project's prime deliverables will be an exploitable prototype monitoring devices for the detection of pathogen (bacteria, viruses and protozoa) in the water supply network.

The project wants to demonstrate the viability of the technology developed by producing prototype devices to meet specific end-user requirement specifications.

The objective of the project is to develop, through the combination of nanotechnology, microsystem technology and biochip technology, a forward looking warning system.

1.2 Contractors Involved

Participants Name	Country	WWW
Lambda GmbH (Coordinator)	AT	www.lambda.at
BHR Group	UK	www. bhrgroup.co.uk
Idea s.r.l.	IT	
Hemosoft	TR	www.hemosoft.com
MikroMikoMed Ltd.	HU	
Water Research Institute Bratislava	SK	www.vuvh.sk
Università di Bologna	IT	www.eng.unibo.it
Budapest University of Technology and Economics	HU	www.ett.bme.hu
Cranfield University	UK	www.cranfield.ac.uk
Steinbeis-Europa-Zentrum	DE	www.steinbeis-europa.de
LioniX B.V.	NL	www.lionixbv.nl
Provenion	DE	www.provenion.de

1.3 Work performed & Methodologies and approaches employed

The DINAMICS project has during the four year project period developed and assembled a prototype device for the detection of pathogens in drinking water integrated with an warning system that will automatically alert authorities through different communication channels. The prototype consists of several modules and its corresponding disposable microfluidic chips and a central control unit.

Requirements and Specifications

At the start of the project “User requirement specifications” and “Functional specifications” that translated these requirements into particular specification for type of pathogens included, response time, sensitivity, reliability, functionality and ease of use were defined by studying literature and employing an advisory board and making engineering trade-offs.

Fluidic Modelling

Modelling the flow and mass transport in microfluidic devices ensures efficient progress in the design and manufacturing of the microfluidic structures. A reduction in the number of the development cycles is expected by using simulation instead relying solely on trial and error.

Several reports have been prepared describing the state of the art in modelling micro- and nanoscale diffusion processes and in modelling techniques to describe diffusion phenomena. A literature review for physical phenomena observed as the operational dimensions of the devices are downsized to nano-scales has been carried out. Based on an initial critical review of literature the most appropriate methodologies for implementing and coupling the relevant tools at both the molecular and macro (continuum) scales were chosen.

A multi-scale modelling toolkit was developed combining a suite of novel coupled and un-coupled Computational Fluid Dynamics and Molecular Dynamics software.

The simulation toolkit has been used to develop and test strategies to guide the design and to optimise the performance and efficiency of the measurement device. A number of alternative improved sensor geometries employing "hydrodynamic focusing" techniques, whereby the channel geometry is optimised to focus the DNA strands into sensor region, increasing hybridisation and hence signal detection. Active Microfluidic systems have been studies. More specifically, several simulations with active acoustic wave sources have been carried out.

Device Development

An in-line-separator for increasing the pathogen concentration was developed based on ultrafiltration. Various options were explored and the final prototype obtained an enrichment factor of ~ 1600 in 30 minutes. As gold standard optimal protocols for target organism enrichment by membrane filtration have been established, that ensure maximum recovery of bacteria and eukaryotic oocysts.

Several approaches for cell lysis to release DNA from pathogen cells were studied and investigated experimentally. Two prototypes of a cell lysis device have been realized combining three different

lyses approaches. A simplified lyses module with a disposable lyses chamber developed and proved functional in lysing bacterial cells.

A prototype of a microfluidic DNA-purification module has been developed and successfully tested. An improvement in the design of the lysis module and a change in the flow control made a continuous, faster and lossless transfer of samples from lysis to DNA-purification module possible.

A prototype on-chip PCR module equipped with a fully automated liquid handling system has been assembled. A new micro-mixer design for a laminar flow regime resulted in a maximum diffusion distance for components to reach complete homogeneity is in the order of 2 µm.

Research in nanotechnological signal enhancement aims at increasing the detection sensitivity by either target or signal amplification. Four nanotechnological signal enhancement methods were studied, employing chemical or enzymatic reactions. Characteristics and limitations of the signal acquisition interface has been studied. Two isothermal DNA amplification methods have been studied for target amplification and can be integrated into the device instead of PCR without changing the system.

The primers necessary for amplification and the probes used for the capture of the target molecules were determined with bioinformatics methods. For optimal DNA probe immobilization on different substrate materials: Gold, Quartz and Polymer robust protocols for optimal surface functionalization had to be developed.

Both electrical and optical methods were investigated for their use in the detection module. Various electrical and electrochemical measurement principle could be implemented on existing electronic measurement boards. Initial measurements indicate that the limit of detection for oligonucleotides is at least 10nM. The new optical detection module has been integrated with a microfluidic driven hybridization chamber implementing a DNA-macroarray. In this set-up chemoluminiscence detection gave detection limit of 180 fM for an oligonucletotides model system.

A Device Control software was developed with a flexible structure allowing to rapidly integrate disparate module controls. Device Drivers for several modules and external components have been created. Web based general purpose contaminant detection and alarming system was established, which successfully integrated a SMS modem for transmitting alarms.

Methodologies & approaches employed

In order to develop a functioning automatic system for the analysis of drinking water for pathogens, experts in several scientific disciplines have been cooperating. The project has been divided into three phases; in the first phase several solutions for each of the sub-functions of the system were explored, in the second phase the functioning solutions were integrated, and in the last phase the system was tested and validated.

The major challenges of such a detection system are:

1, the fast reduction of a large sample volume (100L) to a small analytical volume (< 1ml) that can be processed on a microfluidic platform. This has been addressed by developing an in-line-separator that can reduce the sample volume by a factor of >1000 allowing to introduce a sufficient amount of water into the system and by making use of continuous flow of the total sample volume from one module to the other thereby reducing sample losses to a minimum.

2, the integration of several technologies (nanotechnology, microfluidics, microelectronics, and molecular biology) with often contradicting requirements. A rigorous systems engineering approach was necessary to overcome this difficulties.

Achievements to the current state-of-the-art

Since the start of the project several groups have been working on similar systems for the detection of pathogens in drinking water.

The most advanced device seems to be already in the early stage of commercialization by "Early Warning Inc." a spin-off from NASA's Ames Research Center. It has a similar concept but it differs from the DINAMICS device in several aspects.

The differences are

1, that nucleic acid extraction is performed with magnetic beads, while DINAMICS uses a solid phase to avoid potential clogging of the microfluidic with beads

2, DINAMICS uses DNA as targets which allows to detect all kinds of pathogens (bacteria, viruses and eukaryots) while the competitors system uses only rRNA which excludes the detection of viruses

3, DINAMICS uses a nucleic acid amplification, which has the drawback of extra time and system complexity (even with isothermal methods) but guarantees much higher sensitivity compared to systems without.

4, DINAMICS applies an optical chemiluminiscent based detection process, which is a quite robust and well established technology and also less prone to unspecific signals arising from electrical signal generation.

We thus believe that the work performed within DINAMICS is at the forefront in the field of development for automated early warning systems for pathogen detection in drinking water.

1.4 End Results

The project team produced a prototype lab-on-a-chip platform to quickly, sensitively and simultaneously detect a multitude of pathogens in water.

The system can take water directly from the water supply system (pipes, reservoirs, etc.) and feed it to the analysis platform. Large sample volumes are necessary to guarantee the highest security level for the population.

The detection system consists of five integrated microfluidics based modules (Lysis, DNA-isolation, DNA-amplification, signal-enhancement and detection) that are connected to an alarm system. The alarm system can automatically send warning messages to the authorities, who then can take the appropriate actions.

The time consuming laboratory procedure with five manual procedures that would take at least 10 hours from taking the sample to result will be reduced to a fully automated process that will be completed within 2 hours.

Proof-of-principle tests have been performed for a model organisms on the modul level. The system functionality has been verified but field tests of the whole system were out of the scope of the project.

Disposable microfluidic chips and cartridges have been manufactured and used for validation studies. Simulation tools and rapid prototyping technology have been applied for design and validation.

Impact of the Project

The DINAMICS results demonstrate that it is feasible to implement an integrated and automated warning system for the protection of the civilian water supply network from bioterrorism.

DINAMICS also anticipates that the results of its research work will gain entry into the medical diagnostics of infectious diseases and could revolutionize the methods being used there at present. The innovation has also applications inthe food industry and pharmaceutical testing.

1.5 Project Logo & Website

http://www.dinamics-project.eu

1.6 Coordinator contact Details

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2 Dissemination and Use

Many of the results & the knowledge coming out from the project should be either commercially exploited or used internally in further research activities. Some have been already be partially presented at conferences (general presentations, see section 3) or published in scientific articles (see below), but most of them are still not published (see p24, publication planed). As most of the partners still did not complete the IPR issues concerning results they want to exploit, only the following project result should be published on CORDIS for the moment.

Result "Name": Lyses Device

Result description:	The result concerns a programmable Pathogen-lysis Device which consists in a hardware and a PC software. Latter serves as an interface towards the user, who can adjust 5 parameters of a microbiological sample pre-treatment process serving for the lysis (ie. release of DNA/RNA content) of pathogen / human cells controlled by the hardware. The 5 programmable parameters are: 1. temperature, 2. overpressure, 3. flow rate of sample solution containing the pathogens, 4. flow rate of lysis buffer, 5. time of process The concept of the chemical/physical lysis protocol proposed is suitable for automation or in-line deployment, thus, applicable in several industrial fields related to pathogen detection. The hardware is flexible in mechanical design for customisation according to the needs of a new user and the PC software allows user friendly operation
Level of Development	Laboratory Prototype
Sectors of application foreseen with expected timetable for commercial use:	Microbiology process developers, Microbiological service provider laboratories. Market entry: 2012 Q2
Kind of exploitation and collaborators foreseen:	Commercialisation directly as a research tool (product) to developers and laboratories, or offering it in the frame of further research projects for potential new partners
IPR protection measures planned:	Industrial secret (source code of software and firmware are kept, design files of hardware are kept).
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