# Development of an Integrated MEMS-based DNA Analysis Chip with Active Flow Control Components for Space Applications

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## ABSTRACT

This paper addresses the development of a novel DNA chip with fully integrated functionalities, including sample preparation, amplification, genetic analysis and data processing, coordinated by D'Appolonia in the frame of an international project involving European industries and Research Centers.
State-of-the-art DNA analysis techniques still suffer from time-consuming and labor-intensive procedures, requiring costly and bulky equipment as well as great quantity of costly reagents, taking several hours to deliver results and resulting prone to human errors. The SMART-BioMEMS project aims to develop a micro-device ideally suited for ultra-fast, cost-effective and highly reliable point-of-care genetic analysis. These characteristics make it a potentially ideal candidate for future space applications, such as microbial sampling of internal environment in manned habitats or in-situ genetic analysis of planetary samples, providing fast and accurate information about hundreds of genes simultaneously.

## 1. INTRODUCTION

Future space exploration manned missions require the development of dedicated Life Support Systems (LSS), i.e, all equipment necessary to the astronauts for safely and efficiently performing their physiological and operational activities. Typical LSS include air production and cleaning, water and food supplying, waste recycling, health monitoring and many others.
Up to date long-term manned space missions (such as Mir and ISS) have been limited to Low Earth Orbit (LEO), allowing the implementation of basically not regenerative life support functions where the most part of supplies and consumables is periodically brought from Earth through dedicated additional missions.
However, for space missions beyond LEO, such as the future exploration of Moon and Mars, this will not be possible anymore. When space crews are operating at hundreds thousands of kilometres from Mission Control they need to bring all right tools with them, and cannot afford large quantities of spares.
Because of the cost of every kilogram sent to space, especially high for misions beyond Earth orbits, and the scarcity of on-orbit resources, miniaturization is becoming more and more important. The size and mass reduction of equipment and payloads allows flying a larger amount of material, increasing the overall mission performance, efficiency and reliability. Therefore, future life support space-based systems must be small, have low mass, low power consumption and exercise high degree of control.

## 2. BIOCHIPS APPLICATION IN SPACE

A consistent amount and variety of microbial contaminants (such as bacteria, fungi, viruses, etc.) is envisaged to reside onboard of manned habitats for future space exploration missions. This is not only due to the normal production by human beings, as many types of micro-organisms will be intentionally carried along from Earth in the microbial-based systems currently being developed in Europe (MELISSA [1]), USA (BIO-Plex [2]), Japan (CEEF [3]) and Russia (BIOS [4]) to support astronauts life functions, such as for example food production and waste recycling.
Consequently, several sources of biological contamination are envisaged in a space manned habitat, such as the cabin air (from crew expiration and sweat), gases exhausted from solids treatment systems (e.g., food, plants and fecal residues), the atmospheric condensate in the cabin and greenhouses for space-based cultures, or the wastewater of hygiene, laundry and food preparation (generally indicated as *graywater*).
From Earth laboratory experiments mimicking space flight conditions it is known that typical space environmental factors, such as microgravity, temperature, pressure, aeration, moisture, vibrations, radiations, etc., can induce and select for physiological, metabolic and/or genetic variations in micro-organisms characteristics strongly related to life support issues [5]. Among these can be cited the development of higher growth rate and survival resistance, inhibited production of antibiotics, enhanced virulence and pathogenicity, exhibition of different genetic profiles for example to protein and immuno-reactivity.

However, space environmental conditions simulated in ground laboratories cannot take into account or exactly reproduce all aspects of the real environment of manned modules in space. For example, bacterial cells tested in bioreactors with rotating fluids to simulate microgravity effects are subjected to shear stresses which can alter the observed behaviour, and irradiation experiments performed with single beams mimicking the separate ranges of the complex cosmic radiation spectrum do not guarantee the prediction of the total biological effects by the simple superposition of all spectra components. In addition, long term observations (more than 6 months) of bacteria evolution are not available.
On the other hand, limitations related to space missions such as availability of electrical power, stowage space, crew time, and a general lack of onboard sophisticated analytical equipment and expertise, do not allow for frequent flight experiments opportunities. Consequently, an exhaustive and detailed assessment of the behaviour of microbial populations in closed space-based environments has not been attained yet.
During future space exploration astronauts will live in confined modules at very long distances from Earth, with no possibility of procuring additional emergency means besides those carried along from Earth foreseen at the beginning of the mission, and with great difficulties for an eventual rapid evacuation of a contaminated habitat. In this situation bacteria undergoing pathogenic mutations may jeopardize the well-being of crew, and also materials may be compromised by micro-organisms showing biodegradative and biocorrosive capacities.
Therefore, environmental monitoring of bacterial activity under space flight conditions is of fundamental importance for the early detection and identification of changes in micro-organisms communities linked to medical, environmental or life support issues, and the consequent prompt undertaking of adequate recovery actions. To this respect, the use of innovative biochip technologies, characterised by reduction in mass, size and power consumption, as well as by miniaturized diagnostics enabling the execution of a large number of tests on the same device, promise important benefits in the improvement of future space LSS.

## 3. DESCRIPTION OF THE SMART-BIOMEMS SYSTEM

The aim of the SMART-BIOMEMS project is the design, realisation and testing of a prototype micro-system for molecular diagnostics, capable of providing for a complete integrated genetic analysis on-a-chip, from the introduction of raw biological sample to the accurate determination of single genotypes.
The main objective of the project is related to the fine detection of disease-related genotypes in terms of gene mutations, enabling to identify possible predisposition to diseases such as cancer in individuals or families. Due to its small dimensions and fast analysis capabilities, the SMART-BIOMEMS system will allow focussing of medical diagnostics directly at the point-of-care, avoiding typical delays of laboratory testing and providing clinicians the unique genetic profile of patients to determine the most effective personalised treatment. Additional benefits are envisaged in food safety (crop development, livestock breeding, etc.) and animal health in terms of bacteria, toxins or allergens detection.
The proposed micro-system is the first example of advanced sample Pre-Treatment (PT) integrated on chip with coupled Polymerase Chain Reaction (PCR) amplification and post Single Nucleotide Polymorphisms (SNP) analysis (Fig. 1). The system is provided with pre-treatment capabilities for sample purification through integrated DiElectroPhoresis (DEP) micro-structures, selectively withholding sample cells while removing DNA amplification inhibitors such as haemoglobin. Following cell thermolysis releases high-quality DNA molecules directly in the PCR micro-reactor, where target DNA is replicated to make available enough starting template for the subsequent sensitive and specific analyses. Unambiguous detection of specific DNA mutations or SNP is performed through development of a Primer EXtension (PEX) reaction, based on the extension of oligonucleotide probes by a fluorescence-labelled “terminator” designed to bind the complementary template under investigation immediately adjacent to the mutant position. A separation step is foreseen after the PEX reaction to wash off the unincorporated terminators, thus allowing for highly accurate SNP identification through an imaging fluorescence system.
The entire process, from raw sample to SNP detection, is implemented on-a-chip by addressing latest advances in MEMS (Micro Electro-Mechanical System) technology, possessing appropriate potential to enable complete DNA analysis with increased functionality and performance at reduced costs, especially due to the optimised consumption of costly reagents.

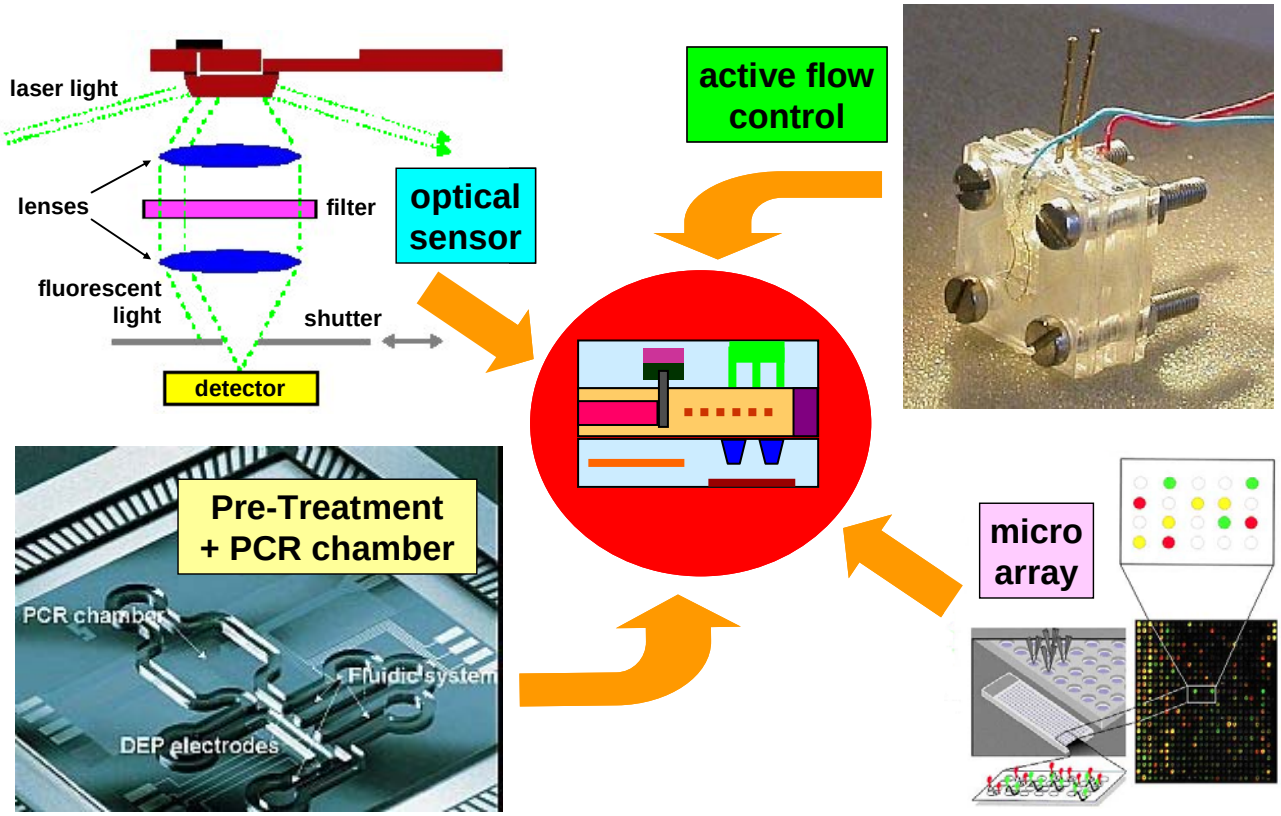


Fig. 1. Schematic composition of the SMART-BIOMEMS system

As the performance of microfluidics-based instrumentation integrated in 'lab-on-a-chip' systems strongly depend on control of fluid motion, the development of the SMART-BIOMEMS system also addresses critical aspects of optimal sample transportation and mixing with reactants. Innovative Active Flow Control (AFC) actuators are integrated on-chip to precisely move minute amounts of DNA sample and reagents in a controlled way, thus maximising the whole system efficiency. An example of such AFC is based on the principle of “electrowetting”, exploiting the variation of surface energies upon applying an electrical potential between a liquid and a solid. This causes a shift in the contact angle, which can be used for transportation of liquid plugs or droplets (Fig. 2).

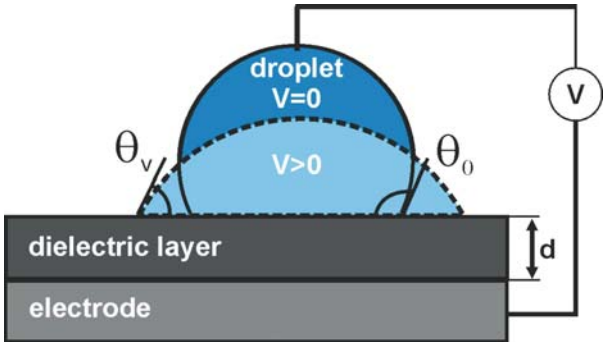


Fig. 2. Electrowetting-based Active Flow Control

Chaotic advection mechanisms are also investigated to promote efficient micro-mixing of DNA sample and reagents in order to overcome the problem of the intrinsically laminar nature of flow in microchannels, where two liquid streams can move side by side for a long time without mixing except for a slow diffusion process.
Furthermore, a micro-optical sensor is specifically designed to interface the system for biological probe loading and fixing on the chip slide, and for detection and imaging of fluorescence signals.
The design of the chip is carried out by advanced optical flow visualization techniques, including micro-PIV (Particle Image Velocimetry) and LIF (Laser Induced Fluorescence), as well as dedicated micro-fluidic CFD models developed

to locally quantify the flow through the micro-channels. Micro-fluidic simulation provide a fundamental contribution to the understanding of the physical phenomena occurring in each functional module, allowing appropriate selection of geometry and materials in order to optimise the most critical parameters of involved processes, thus leading to significant improvement of the system performance.

### 3.1 Sample Pre-Treatment

Sample pre-treatment is an essential step in the analysis of biological materials, as they normally contain many parts not relevant for the performed analysis, which may significantly reduce the efficiency of the following amplification process. An efficient method for sample pre-treating is the use of a selective DEP filter to remove unwanted components from DNA to be replicated (template DNA). The DEP process induces polarisation in uncharged biological cells through the application of an inhomogeneous electric field, the resulting force depending on the difference between the internal properties of the cell and the surrounding fluid (Fig. 3).

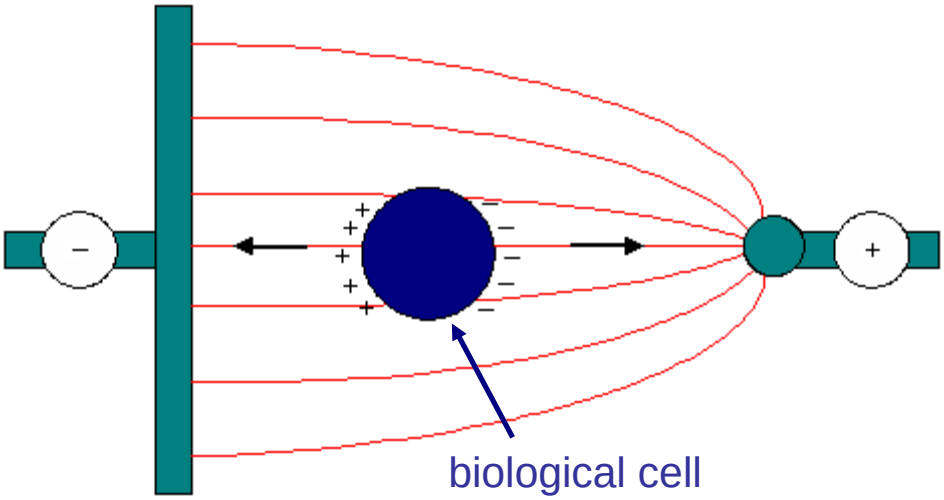


Fig. 3. DiElectroPhoresis (DEP) principle

The DEP filter is designed to attract the cells to the electrodes, while contamination material is flushed out. Purified cells are then treated by thermolysis to release high quality template DNA as a ready sample for the following amplification step. Integration of DEP-based pre-treatment with the PCR thermocycling module allows on-chip purification of samples, thus minimizing cells loss and reducing the amount of external handling before template amplification.

### 3.2 Sample Amplification

The analysis of genetic material requires the amplification of template DNA to compensate for the low concentration of DNA sample to be investigated. This task is accomplished by Polymerase Chain Reaction, an enzymatic method to exponentially replicate specific regions of template DNA template through repetition of thermally controlled reactions.
A PCR thermocycle consists of three main steps (Fig. 4). DNA sample is firstly melted to separate the complementary strands (denaturation). The mixture is then cooled allowing PCR primers to bind to the single-stranded DNA (annealing). Finally, PCR primers are extended by the polymerase reaction (extension), thereby duplicating the desired target site. The efficiency of PCR amplification is optimised by thermal cycling control through on-chip integration of heaters and temperature sensors.
The integration of a PCR module into the micro-system allows to overcome the key limitations of conventional amplification methods, including slow thermal transfer and large use of costly reagents.

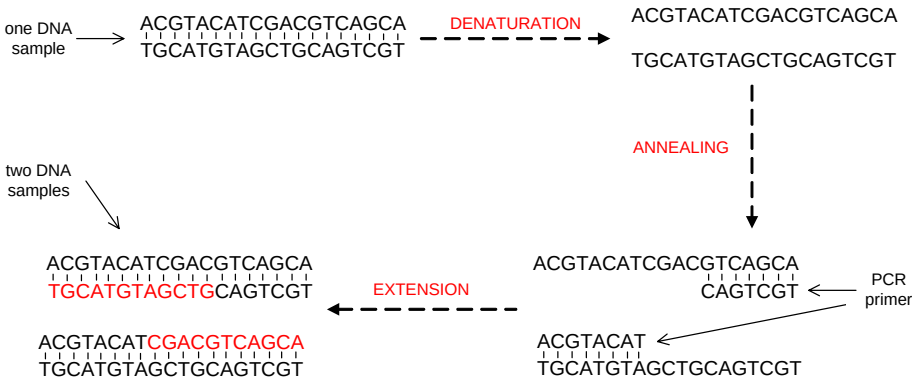


Fig. 4. PCR thermocycle

### 3.3 Micro-Array Genotyping

The microarray-based genotyping module, designed for quantitative SNP analysis, is a collection of miniaturised test sites arranged on a solid substrate, allowing many biological tests to be performed at the same time to achieve higher throughput and speed than traditional tools. It is based on cyclic single-base primer extension reactions (mini-sequencing), in which detection primers carrying 5'-tag sequences bind to the template strands directly adjacent to the SNP position, and are extended with fluorescence-labelled dideoxyNucleotide (i.e., the four bases of DNA) TriPhosphates (ddNTP) by DNA polymerase. The reaction products are captured on the module substrate by hybridisation to oligonucleotide probes complementary to the tag sequences of the primers, immobilised on the slide in an "array of arrays" configuration.
Fig. 5 illustrates the reaction process. Step 1 involves primer hybridisation to the template strand, in step 2 DNA polymerase extends the primer by one fluorescently labelled ddNTP, and step 3 allows primer annealing to the immobilised oligonucleotide probe.

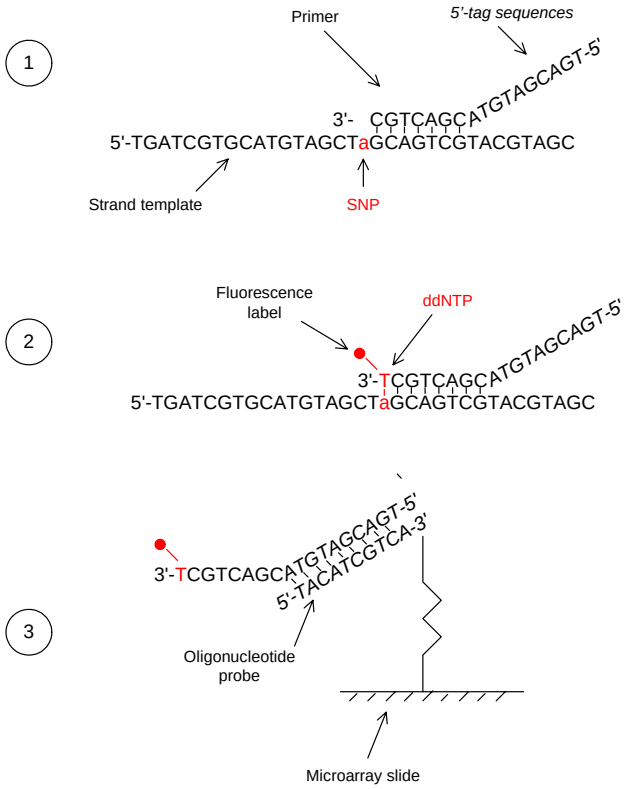


Fig. 5. Mini-sequencing process

### 3.4 Optical Biochip Reading

After hybridisation and washing, the genotype is revealed by scanning the microarray slide through an excitation light source (diode-laser) to measure the fluorescence or chemiluminescence signal intensities in each test site (spot). A CCD-based system, including micro lenses, is developed to capture and process images showing spot intensities for the SNP of interest.
The system is also designed to realise the immobilisation of the oligonucleotide probes on the chip slide by local photo activation (Fig. 6). The light for the activation, emitted by the diode laser, is focused through the micro lenses on the spots in order to allow for a very precise alignment of the immobilised probes.

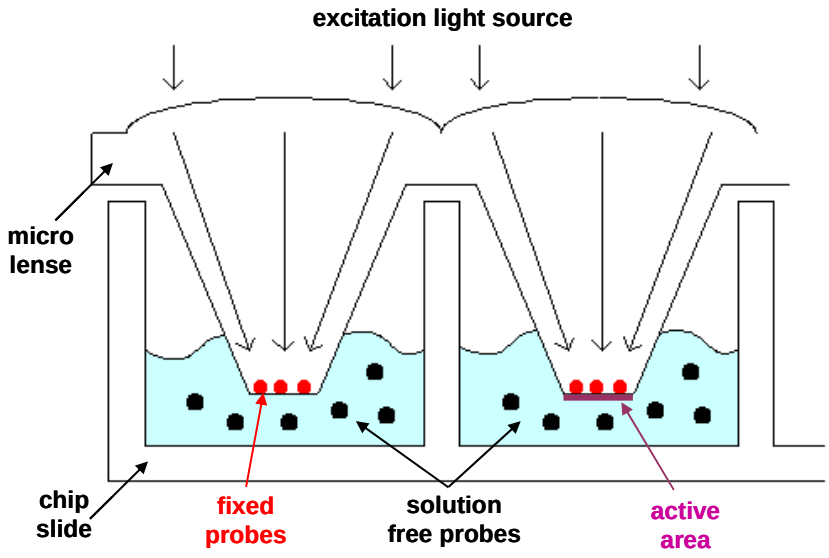


Fig. 6. Local photo activation for probes immobilisation

## 4. CUSTOMISATION OF THE SMART-BIOMEMS SYSTEM FOR SPACE APPLICATIONS

Thanks to its modularity and customisability, the SMART-BioMEMS system shows a great potential to enable conducting genomic analysis of any pathogen agents in a space habitat, including microbes, bacteria and contaminants.
The system relies on complex microfluidic physics to move liquids containing samples and reagents to and from holding regions. In order to adapt the proposed technology for application in space, the complexity of fluid physics in microgravity must be understood and incorporated into the system design. To this respect, help can also be found in data and knowledge gained from macro-scale fluid experiments in space, whose results are not contaminated by the effect of gravity In particular, the relative roles of surface tension physics and multiphase flows, which are expected to dominate in space-bound MEMS devices, must be investigated in detail.
A specific space-based potential application of the SMART-BioMEMS system is related to the detection of microbial contaminants for air quality monitoring in manned habitats. One of the most important aspects to be addressed to fulfil this task is the integration of the system with a portable air sampling device to allow for the detection of aerosolised spores in a closed environment. An innovative bioaerosol sampler capable of capturing airborne particles of biological origin like pathogen agents for subsequent analysis is one of the most promising solutions to be investigated. The sampler shall be specifically designed to guarantee appropriate performance in terms of collection efficiency. In particular, such device shall possess the ability to trap microorganisms into its inlet and transfer them to the collection medium, ensuring at the same time survival or biological activity of bioaerosol particles during sampling.
Further efforts will be required to develop customised purification, amplification and detection procedures, and design specific probes to bind to those complementary DNA base sequences that are unique to each target microbe. The needs of increased automation level will have to be addressed in order to minimise the requirements of human intervention.
The above developments will allow significant advances on the way of progressively increasing the use of biochips in space missions. Due to the enormous benefits they can bring to the safe and efficient accomplishment of human activities in space, a vision can be considered to install onboard spacecraft as much as possible of the diagnostic or processing capability of a state-of-the-art biotechnology laboratory and the medical instruments of an Earth hospital [6].

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