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EXECUTIVE SUMMARY REPORT

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ESA STUDY CONTRACT REPORT

ESA Contract No 4000107911/13/NL/AF	SUBJECT INTEGRATION OF OPTICAL DETECTION IN MICROFLUIDICS SYSTEMS FOR SPACE EXPLORATION MISSIONS	CONTRACTOR KAYSER ITALIA SRL
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ABSTRACT: This document describes the activities and the findings performed in the frame of the ODET-µ (Optical DETection in Microfluidics) project by Kayser Italia, a SME enterprise.

In this project, technologies for the integration of optical components were reviewed to define the “state of the art” in the field of integrated optics for detection on Lab On a Chip devices (LoCs).

LoCs are microfluidics based systems aiming at the miniaturization onto a single substrate (chip) of several functionalities that typically would require an entire biological laboratory.

Although only few commercial devices are in the market, LoC is claimed as a revolutionary technology in the field of Life Sciences being capable of triggering research and supporting point of care diagnostics.

This is due to the following advantages: high sensitivity, speed of analysis, low sample and reagent consumption, and measurement automation and standardization. Overall cost reduction would pave the way to LoCs into every day laboratory practice.

Presently, optical detection is the main limitation of LoCs. As a matter of fact, it is performed “off-chip” frustrating the advantages of miniaturization.

Results of the review were presented and improved in a meeting with representatives from both Academia and Industry involved in the field. Outcomes show that new technology developments on integration of optical components on Lab On a Chip are currently underway.

In this frame optofluidics is an emerging field of research.

Traditional integration technologies (such as Silica on silicon planar technology, Ion-

exchange in glasses, Soft-lithography in polymers) and novel technologies (Femtosecond laser micromachining, Liquid core waveguides, Plasmonic detectors, Optical microresonators) are reviewed and reported.

Detection methods for biological assay are critically discussed for LoC application with respect to the bio-parameters of interest of ESA.

A trade-off among available technologies for the integration of optical detection components on LoC has been carried out. Results showed that integration of optical elements on LoC is feasible.

Silica on silicon planar technology is the candidate technology to be involved in the frame of space missions. Possible application of this technology are presented.

The boundary of integration of optical components is discussed and a hybrid approach, based on a partial integration, is proposed as a reliable set-up.

Intricacies on bioassay development in microfluidics are a main limiting factors for the development of LoC based devices. Fluorescence based assay is the most effective method to be used for biomonitoring, health care, and research application.

A concept for the integration of solid state sensor, capable of single photon counting, for fluorescence detection is proposed as a prospective for a new development in this field with unmatched sensitivity.

Noteworthy, the field of application of such technology can be extended to Biomonitoring, Health Care, and Life Science research applications.

The work described in this report was done under ESA Contract. Responsibility for the contents resides with the author or organization that prepared it.

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EXECUTIVE SUMMARY REPORT

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1 INTRODUCTION

The possibility to control chemical and biological phenomena in miniaturised fluidic system represents a new frontier where many different disciplines such as microfluidics, electrochemistry, and optics are involved.

In the frame of space activities, technology development for applications in the field of Life Sciences is frequently required: investigations in Astrobiology and Exobiology, planetary exploration missions, and support for astronaut's health and wellbeing are presently underway.

The possibility to shrink a whole laboratory on a miniaturized device is the aim of a large and active scientific community. So called Lab On a Chip (LoC) devices are capable of operating all the actions performed in a laboratory granting for a "Sample to Answer" work flow.

Self-standing and reliable Lab on Chip devices would have a strong impact on space based application due to:

- *reduced dimension and power consumption;*
- *higher analytical performances;*
- *portability.*

In line with the requirements and targets of space activities in the field of (but not limited to) space life science in robotic exploration missions, and manned long term space exploration missions, LoC technology potentially is an ideal candidate due to the striking reduction of resources required compared to standard laboratory procedures.

Presently, the main limitation on LoC technology, especially in view of space applications, relies on detection system.

1.1 AIM

The ODET-µ project aimed to define the state of the art in integrated optical detection and the involvement of integrated optical technologies in the frame of a space mission.

1.2 ACRONYMS AND ABBREVIATIONS

Acronym	Description
CCD	Charge-coupled device
LIF	Laser Induced Fluorescence
LOC	Lab-On-Chip
LOD	Limit of Detection
PCR	Polimerase Chain Reaction
PLC	Planar Lightwave Circuit
PMT	PhotoMultipliers Tube
qPCR	Quantitative Polimerase Chain Reaction
RT-PCR	Real Time Polimerase Chain Reaction
S	Successfull
SPAD	Single Photon Avalanche Diode
TV	Threshold Value
US	Unsuccessfull
OV	Overall Value

Table 1: acronyms and abbreviations.

1.3 APPLICABLE DOCUMENTS

AD#	Doc. Number	Issue	Date dd.mm.yyyy	Title
AD01	AO6925-ws00pe	1/0	08th July 2011	Integration of optical detection in microfluidic systems for space exploration missions Appendix 1 to AO/1-6925/12/NL/AF
AD02	AO6925-cc00pe	1/0	08th July 2011	Appendix 2 to AO/1-6925/12/NL/AF
AD03	AO6925-tc00pe	1/0	08th July 2011	Appendix 3 to AO/1-6925/12/NL/AF
AD04	AO6925-li00pe	1/0	08th July 2011	Appendix 4 to AO/1-6925/12/NL/AF
AD05	AO6925-cl01pe	1/0	10th August 2012	
AD06	AO6925-cl02pe	1/0	21st August 2012	
AD07	AO6925-cl03pe	1/0	27th August 2012	
AD08	AO6925-cl04pe	1/0	13th September 2012	

AD#	Doc. Number	Issue	Date dd.mm.yyyy	Title
AD09	AO6925-cl05pe	1/0	27th September 2012	

Table 2: applicable documents.

1.4 REFERENCE DOCUMENTS

RD#	Doc. Number	Issue	Date dd.mm.yyyy	Title	Reference	Author
RD01	Anal. Chem. 2002, 74,2637-2652		2002	Micro Total Analysis Systems. 2. Analytical Standard Operations and Applications		P. A. Auroux et al.
RD02	Nature, 442, 368 (2006)		2006	The origins and the future of microfluidics		G. M whitesides
RD03	Nature, 442, 381 (2006)		2006	Developing optofluidic technology through the fusion of microfluidics and optics		D. Psaltis
RD04	J. Opt. A 11, 034015 (2009)		2009	Laser microfluidics: fluid actuation by light		J.P. Delville et al.
RD05	IBICA-ESASOW-0001 Iss. 1 rev. 4, 28/5/2010		2010	Statement of Work: Immuno-Biochemical Analyser on ISS phase A/B, Annex B: Parameters to be considered		
RD06				Human Research roadmap http://humanresearchroadmap.nasa.gov/		
RD07	TECSHS/5551/MG /ap		2008	Technology Readiness Level Handbook for Space Applications		M. Guglielmi
RD08	ODET-RP012	1/0	29/08/2013	Report On State Of The Art Of Optical Detection In Microfluidics		M. Vukich

RD#	Doc. Number	Issue	Date dd.mm.yyyy	Title	Reference	Author
RD09	ODET-TN010	1/0	08/10/2013	Contents of the open review meeting		M. Vukich
RD10	ODET-TN016	1/0	22/10/2013	Parameters for optofluidics technologies trade-off		M. Vukich
RD11	ODET-RP01910	1/0	20/02/2014	Trade-Off on Integrated optical detection technologies and mission scenario		M. Vukich
RD12	ODET-RP02110	1/0	26/02/2014	Integration of optical detection In microfluidics system for space missions		M. Vukich

Table 3: reference documents.

2 LAB ON A CHIP AND OPTICAL DETECTION

Lab-on-chips (LOCs) are microsystems aiming at the miniaturization onto a single substrate of several functionalities that typically would require an entire biological laboratory (Fig. 1 left panel). LOCs use networks of microfluidic channels to transport, mix, separate, react and analyze very small volumes (micro- to nanoliters) of biological samples (Fig. 1 right panel). The main advantages of the LOC approach are high sensitivity, speed of analysis, low sample and reagent consumption, and measurement automation and standardization. Applications of LOCs range from basic science (genomics, proteomics and cellomics), to chemical synthesis and drug development, high-throughput medical and biochemical analysis, environmental monitoring and detection of chemical and biological threats. Thanks to the miniaturization and integration afforded by LOCs, the life sciences are undergoing a revolution similar to that triggered by integrated microelectronic systems, which gave birth to the Information Society.
Several substrate materials are used for LOC fabrication, including silicon, glass and polymers. Although polymers have the advantages of a very low cost and of the simplicity of microchannel fabrication by molding or embossing, glass is still the material of choice for many applications due to the following benefits: it is chemically inert, stable in time, hydrophilic, nonporous, optically clear, and it easily supports electro-osmotic flow. In particular, the choice of fused silica as the basic material adds to the previous advantages a very high optical transparency down to the UV range and a very low background fluorescence. In addition, well established microfabrication processes, based on photolithography and wet/dry etching, are available for this glass.

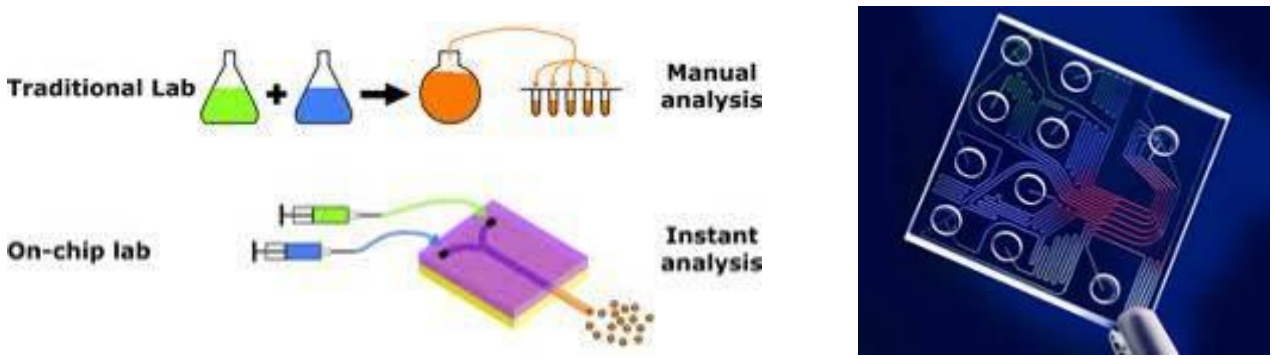

Figure 1: LoC concept vs traditional lab.
Left panel: schematic illustration of the LOC concept.It is compared to a traditional biochemical laboratory. Right panel: example of a LOC device manufactured in glass.

Presently optical detection is performed Off-Chip, frustrating most of the advantages of miniaturization.

The optical detection methods mostly used in combination with microfluidics can be classified in the following categories:

- UV/visible absorption spectroscopy
- Laser induced fluorescence (LIF)
- Refractive index sensing

UV/visible absorption spectroscopy, which measures an absorption spectrum by recording the light transmitted by the sample as a function of wavelength using a spectrophotometer, is the most straightforward method and is a widely used technique in analytical chemistry. It allows to quantitatively identify the composition and the concentration of the sample. As an example, it can be used to monitor biomolecules (DNA and proteins) separated by capillary electrophoresis by monitoring their strong absorption bands in the UV. The main strength of absorption spectroscopy is its simplicity; its problem, when combined with microfluidics, is the limited thickness of the channel, leading to a small path-length traversed by the sensing beam and consequently to poor sensitivity.

Laser induced fluorescence (LIF) detection is a very powerful approach thanks to its background-free nature (the detected light is at a different wavelength with respect to the excitation light) allowing very high sensitivities, with limit of detection (LOD) down to the single-molecule level[25]. An ensemble of molecules in the microfluidic channel is resonantly excited either by a laser or by a LED and their fluorescence is detected after spectral and spatial filtering. To achieve high sensitivities, it is necessary to carefully suppress stray light and autofluorescence either from the LOC material (especially in the case of polymers) or from spurious molecules in the sample. Since most biomolecules do not fluoresce, it is necessary to add fluorescent labels (dye molecules or semiconductor nanocrystals) using well established and highly selective chemical reactions. Fluorescence is the favourite detection method in LOCs due to its advantages, such as high sensitivity and specificity, since one can design fluorophores that specifically bind to a desired molecule. However, fluorescent dyes are expensive and sometimes toxic, and the necessary labelling steps add complication to the microfluidic circuit. In addition, the labeling processes may interfere with the function of a biomolecule or living cell, or may prevent on-chip chemical reactions from taking place. For this reason, label-free detection techniques based on refractive index sensing are highly in demand.

Refractive index sensing usually employs interferometers, which split the light beam into two paths (sensing path and reference path) the outputs of which are then recombined. The sensing path interrogates the volume of interest in the LOC, either directly by crossing it or indirectly by its evanescent wave; the presence of an analyte induces a refractive index change and a phase shift in the sensing beam, which modifies its interference pattern with the reference beam. The presence of the reference beam eliminates all the common-mode fluctuations due to, e.g. temperature changes or intensity changes. Several interferometer designs have been demonstrated, including Young and Mach-Zehnder interferometers. The main strength of refractive index sensing is its label-free nature, allowing to monitor a molecule in its native, unperturbed form; however the achievable LODs are not as good as those achieved with LIF and in addition the technique is less

specific, since it only senses a refractive index change, so that it needs to be combined with some other selective method, such as an antibody/antigen reaction. A very recent and promising approach to refractive index sensing exploits surface plasmon polaritons (SPP) resonances, which occur at the interfaces between a metal surface and a dielectric. The spectral position of such resonance is very sensitive to the refractive index of the dielectric medium, e.g. the liquid sample under analysis. By functionalizing the metal surface with an antibody, the presence of an antigene molecule in the analyte specifically binding to the antibody will modify the refractive index at the interface. This induces a shift in the SPP resonance which can be sensitively detected by monitoring the metal reflectivity.

3 REVIEW OF INTEGRATED OPTICAL DETECTION TECHNOLOGIES

A review on technologies for integration of optical components was carried out. Particularly, attention was paid on a new active field of research: **Optofluidics**.

3.1.1 INTRODUCTION TO OPTOFLUIDICS

The integration, on the same substrate, of optical and microfluidic components has far-reaching scientific and technological implications, that go beyond the specific application of sensing in LOCs. To define this new field of research, the terms “optofluidics” has been recently introduced in the scientific literature.

Optofluidics exploits the synergy of optics and fluidics for the realization of completely new functionalities, such as optical elements whose optical properties can be tuned through fluid replacement or modification, resulting in reconfigurable and adaptive microphotonic devices or microfluidic devices with integrated optical sensing and manipulation.

3.2 REVIEW OF TECHNOLOGIES

A review on presently available technologies for the integration of optical components on chip was carried out.

On the whole, reviewed technologies can be divided in two main classes:

- traditional methods (standard and well-consolidated) that are based on the fabrication of integrated optical circuits in glasses and polymers and their application to on-chip detection schemes;

- novel optofluidics based methods, relying on technological advances that have occurred during the last decade, that are involved in on-chip sensing of fluidic samples.

Traditional methods are:

1 silica on silicon planar technology:
fabricates optical waveguides and photonic devices by deposition, photolithography and etching of transparent oxides on a silicon substrate;

2 ion-exchange in glasses:
fabricates optical waveguides and photonic devices by the inclusion of potassium or silver ions in specific regions of a glass substrate, defined by suitable masks, to create positive index contrasts;

3 photo & soft-lithography in polymers:
fabricates optical waveguides and photonic devices by the initial production of specific patterns on a stamp, that are then transferred onto plastic replicas.

Novel methods are:

1 femtosecond laser micromachining:
employs focused femtosecond pulses capable of nonlinear material modification, enabling to produce both waveguides and microfluidic channels;

2 liquid core waveguides:
these waveguides employ fluids as physical means behaving as the core of the waveguide;

3 plasmonic detectors:
these structures employ thin and functionalized metallic nanostructures presenting sharp plasmonic resonances, capable of enabling sensitive and reliable sensing of analytes through the refractive index induced shift of these resonances;

4 optical micro-resonators:
these structures employ resonant optical microcavities presenting very sharp peaks, which can be used for very sensitive label-free detection thanks to their refractive index induced shifts.

4 STATE OF THE ART ON INTEGRATED OPTICAL DETECTION

The review of the literature performed led to the definition of the state of the art in the development of integrated optical detection technologies. Such technologies are capable of providing optical detection of bio-parameters.

The majority of parameters of interest for space application (mainly parameters related to healt-care, see Annex 1) are optically detectable (92%). Currently, their detection is performed off-chip and it is based on well known protocols and measurements.

Presently, the detection of such paramenters is based on immune assays, largely used both in research and diagnostics laboratories. Detection is mainly based on spectrophotometric measurements of optical density.

Our results based on the review of the scientific literature shows that **on-chip optical detection** of such parameters **is fairly limited**. Moreover, most of the information relies only on a single published paper.

It is worth nothing, that integrated optical detection on chip largely involves fluorescence measurements.

There are a number of consideration that supports fluorescence detection as the best detection methods:

- allows for lower Limit of Detection (LOD);
- it is widely used in Bio-assay:
  - immune test (ELISA and derivatives);
  - nucleic acid amplification (PCR, qPCR, NASBA);
- protocols are world wide used.

Refractive index sensing is limited by:

- higher LOD;
- sophisticated alignment of optical components;
- requires specific tests to be used/developed being the measurement not specific.

Nevertheless, being refractive index sensing a label free detection it has the potential advantage of a reduced involvement of reagents.

Most of developments in the field of microfluidics are limited by the reduced number of tests that can be performed by that microfluidics platform.

Presently, downscaling an immune assay to a microfluidic environment takes about 2 years.

If the availability of downscaled bio-assays in microfluidic environment increase, integrated optical detection on-chip allowing the measurements of fluorescence will provide a powerful tool for detection, especially for astronaut's health and biomonitoring.

Coming to biomonitoring an excellent example is given by the ESA MIDASS instrument. Universal detection of microbial forms is based on nucleic acid amplification and fluorescence measurement. The procedure takes place in a fluidic cartridge containing all the chemicals required by the protocols. Volumes are compatible to microfluidics: each test requires 5 µl.

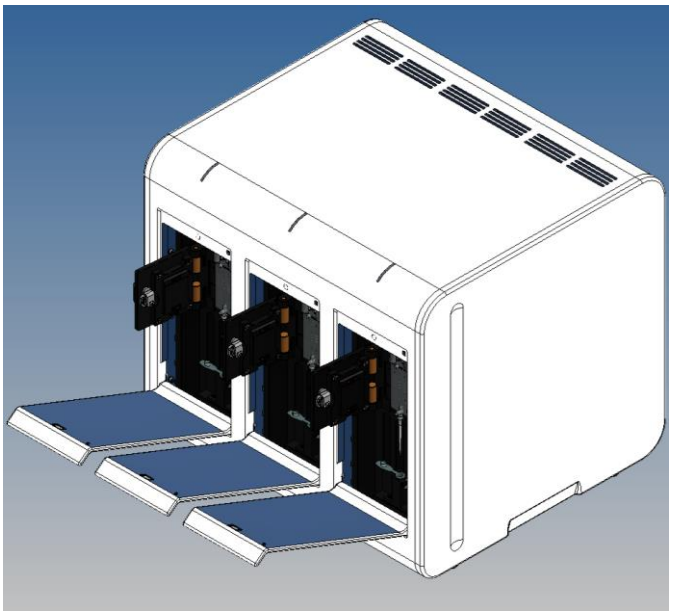

Figure 2: MIDASS detection modules.

4.1 BOUNDARIES OF INTEGRATION

Generally speaking, integration in LoC devices is a driving concept. On the other hand, integration can lead to a dramatic increase of complexity affecting the system negatively.

Referring to biological parameters detection, sample processing (i.e. the isolation of target molecules) is a main factor capable of increasing LoC complexity. Operating sample preparation outside the LoC mitigates its complexity. Nevertheless, in some cases sample preparation and detection can be provided by the same microfluidic device, e.g. like separation of plasma and serum from blood samples and detection on a microfluidic minidisc.

Fluidics can be reduced taking advantage of magnetic beads. Magnetic beads support sample detection too, being a proper physical substrate for surface chemical modification.
The kind of surface for optical detection (beads surface, or planar surface) used for sample localization represents a first requirement for optical detection.

Presently, hybrid technology based on docking station chip interface seems to be capable of overcome technology limitations for a fully integrated LoC device.
Docking station can support a number of components/functionalities relieving LoC design. As a matter of example fluidics activator, sample preparation, thermal interface, optical system subunits can be part of the docking station.

5 TECHNOLOGY TRADE-OFF

Trade-off of the technologies reviewed cannot be carried out focusing on biological parameters.

This is due to:

- The small overlapping between biological parameters of Interest of ESA and those which are presently detectable with integrated optofluidics techniques;

- As optofluidics techniques are noverl, information on the detection of biological parameters is often failrly limited to a single published reference;

Thus, a technology trade off focused on a list of biological parameters, e.g. naming specific molecules to be detected would bias the trade-off.

Trade-off of integrated detection technologies was based on their ability in detecting fluorescence, absorbance and refractive index change signals (in fact a single technology can be used to detect difference signals).
Last but not least, biological detection is primarily bound to the biological assay that can be designed so to allow for different detection methods (absorption spectroscopy, fluorescence and refractive index sensing).

5.1 REVIEWED TECHNOLOGIES AND SIGNAL DETECTION

In general the traditional fabrication methods (silica on silicon, ion exchange and lithography in polymers) are quite flexible, as they allow detecting different optical signals (absorbance, fluorescence, refractive index change).
The same is true for two of the innovative methods, femtosecond micromachining and liquid core waveguides.
Plasmonic detectors and microring resonators, on the other hand, are intrinsically sensitive only to refractive index change, for which they achieve a sensitivity that significantly surpasses that of standard technologies.

Technology	Fluorescence detection	Optical density detection	Refractive index change detection
Silica on silicon	YES	YES	YES
Ion exchange	YES	YES	YES
Lithography in polymers	YES	YES	YES
Femtosecond micromachining	YES	YES	YES
Liquid core waveguides	YES	YES	YES
Plasmonic detectors	NO	NO	YES
Optical microresonators	NO	NO	YES

Table 4: overview of the signal detection capabilities of different optofluidic technologies.

As a matter of example a trade-off table is reported below.

	General parameters	Technology		
	Technology readiness level			Notes
		S/US	Value	Short explanation of value
General parameters	Present maturity level			
	Performance in signal detection			
	Complexity of sample and sample preparation			
	Multiplexing capabilities			
	Risk factors			
	Technological load			
	Technology robustness			
	Technical budgets			
	Number of potential applications			
	OV			
	TV (60%)			
	Trade-off outcome			
Specific parameters	Crew risk reduction 1-Sample Kind - Type - Family - Size 2- Relevance of diagnostic output; 3- Analysis time; 4- Reduced crew involvement in risky activities;			
	Provision of novel capabilities			
	Level of containment			
	Chemical hazards (toxicity and safety aspects)			
	Breakthrough technology			
	OV			
	TV (60%)			
	Trade-off outcome			

Table 5: parameters for technology trade-off.

The trade-off process is based on two stages: Stage 1: an arbitrary scale of values from 1 to 10, being values 6≤X≤10 considered successful, is given per all Generic Parameter to each technology. The ∑ of the values for all the Generic Parameters (criteria) considered will provide the overall value (OV) per each technology. Technology trade-off will be carried out referring to a threshold value (TV) equal to 60% of successful values. Technology Readiness Level (TRL) is assigned as for [RD7- Technology Readiness Level Handbook for Space Applications]. As low values are expected TRL is not included into the calculation of the OV. The TRL value complements technology trade-off. A technology having an OV≥TV will undergo stage 2.
Stage 2: an arbitrary scale of values from 1 to 10, being values 6≤X≤10 considered successful, is given per each Specific Parameter. The ∑ of the values for all the Specific Parameters (criteria) considered will provide the overall value (OV) per each technology. Technology trade-off will be carried out referring to a threshold value (TV) equal to 60% of successful values.

5.1.1 TRADE-OFF SUMMARY TABLE

	General parameters		Specific parameters		Trade-off matrix	
	S/US	Score	S/US	Score		
Silica on silicon (PLC)	Y (9/9)	76	Y (5/5)	43 (119)	Y;Y	**Passed**
Ion exchange	Y (6/9)	61	N (2/5)	31 (92)	Y;N	Not passed
Lithography in polymers	N (4/9)	53	Y (5/5)	32 (85)	N;Y	Not passed
Femtosecond micromachining	Y (8/9)	67	Y (5/5)	39 (106)	Y;Y	**Passed**
Liquid core waveguides	N (5/9)	56	Y (4/5)	36 (92)	N;Y	Not passed
Plasmonic detectors	Y (8/9)	69	Y (5/5)	37 (106)	Y;Y	**Passed**
Optical microresonators	Y (9/9)	75	Y (5/5)	38 (113)	Y;Y	**Passed**

Table 6: trade-off summary table.
S Succesfull, US unsuccesfull. Y In compliance with General/Specific Parameters, N Not in compliance with General/Specific Parameters. Maximum score: 9/9 Succesfull, 90/90 Score, 5/5 Succesfull, 50/50 (140/140).

Trade-off results were compared vs instrumentations having integrated optical components on chip available in the market. Moreover, as most of the applications of optofluidics technologies are at laboratory level, in order to define reliable technical budgets commercial bench instrument and a commercial portable were taken as reference.

Budgets summary table			
Overall budgets	**Mass**	**Power**	**Volume**
Chip	2-3 grams	n.a.	40×20×2 mm^3
Cartridge	20-30 grams	n.a.	50×30×15 mm^3
Docking station	300-400 grams	n.a.	100×50×30 mm^3
Driver Unit (standard)	5-50 kg	20÷50 W	400×300×50 mm^3
Driver Unit (advanced)	0.5-3 kg	<0.5÷5 W	<200×150×30 mm^3

Table 7: technical budgets of the different components of an optofluidic device.

On the whole, all the optofluidics technologies examined have valuable features for at least one of the following:

- UV/visible absorption spectroscopy;
- Laser induced fluorescence (LIF);
- Refractive index sensing.

Nevertheless, trade-off results indicate:

- Silica on silicon (PLC);
- Femtosecond micromachining;
- Plasmonic detectors;
- Optical microresonators.

as most performing technologies and thus candidate technologies for space applications.

6 POTENTIAL UTILIZATION IN A SPACE MISSION

Although commercial instruments are available on the market, optofluidics is a new field of research. In view of a potential utilization in a space mission, further considerations are relevant in order to identify the best technology.

Femtosecond laser micromachining
Particularly, femtosecond laser micromachining can provide processing in hard materials or 3D channels or structures that cannot be achieved otherwise. This technology is still in the research phase, but well matured for specific application (modification of commercial chip for instance).

Nevertheless, this technology does not support biological detection in biological tests where functionalization is required. In fact, LOD in femtosecond laser micromachining for label-free detection is not as good as for fluorescent detection and lower of competitor technologies. Once more, label-free detection would simplify overall set up due to a minor involvement of fluids and related handling. Moreover, as biological detection in space is limited by the limited shelf-life of many reagents, label-free approach is preferable.

Plasmonic detectors and microresonator
Among the novel methods examined, optofluidics sensor based on plasmonic detectors and optical microresonators passed. Optical microresonators, scored 113 while plasmonic detectors 106.
Commercial devices are also available based on both sensors. Nevertheless, they are bulky (overall mass around 50 kg) and optics, especially for plasmonics based sensor

requires light propagation in free-space and therefore alignment is very critical. This aspect hinders the device portability.

Silica-on-silicon (PLC) is based on very mature semiconductor industry technologies and it is the widest used technology in the field and the most robust.

Integration of optical elements into LoC device is proven and effective.

This technology, due to its robustness, offers high possibility of integration of optical element on LoC especially waveguides. It has to be taken into account for possible involvement in space.

The possible spatialization of a commercial device, the Q-sense instrument by PLC diagnostics, for point of care testing and biomonitoring based on PLC technology is considered feasible.

Again the panel of test that can be performed is limited to:

Q-SENSE targets diagnostic applications:

- Acute Myocardial Infarction (AMI)
- Cardiac marker (Troponin I & T, BNP, D-Dimer, hFABP & Myoglobin)
- Infectious Diseases (*C. Difficile*)
- Mild Traumatic Brain Injury (S-100B, GFAP & UCH-L1, mTBI)
- Pathogens & Toxins screening (*C. Difficile* and *C. Difficile* toxins A & B)
- Cytokins (IL-1β)
- Circulating Tumor Cells

It is claimed to be extended to rapid Gene-Expression panels that are fundamental for **biomonitoring** based on nucleic acid amplification.

7 DEVELOPMENT OF A COMPACT ANALYTICAL DEVICE BASED ON MICROFLUIDICS AND SINGLE PHOTON DETECTION FOR SPACE APPLICATIONS

Presently, a technological solution for full integration of optical detection in microfluidics is not available. Moreover, the overwhelming number of applications in the field of Life Sciences (including diagnostics and health care) and heterogeneity of sample kinds to be investigated prevent from the development of “inclusive” technology.

As a matter of fact, successful technological developments in microfluidic have been achieved but for very specific applications.

In the following the concept for a compact analytical device is proposed.

7.1 SINGLE PHOTON DETECTION

The term SPAD defines a class of photodetectors able to detect low intensity signals down to the single photon and to signal the time of the photon arrival with a temporal resolution of few tens of picoseconds exploiting the photon triggered avalanche current of a reverse biased p-n junction.
SPADs offer the advantages of both PMTs and CCDs: being solid state devices, they are easier to combine in compact parallel systems then PMTs, offer higher photon detection efficiencies and require less power to operate. On the other hand, SPADs present a faster response time than CCDs, so are more suitable when fast signal are to be detected, for example in FCS (Fluorescence Correlation Spectroscopy) measurements.

7.1.1 ANALYTICAL DEVICE

Basically, the device is a fluorescence detector.

The device is composed of:

Microfluidic platform
- Excitation and emission waveguides;
- Cell culture chamber;
- Reservoir;
- Measurement chamber;

Detection system
- SPAD camera (integrated into docking station);
- Emission and excitation filters (integrated into docking station);
- Collimating lenses (integrated into docking station);
- Light source (integrated into docking station).

Docking station

- Control electronics;
- Fluidic actuators.

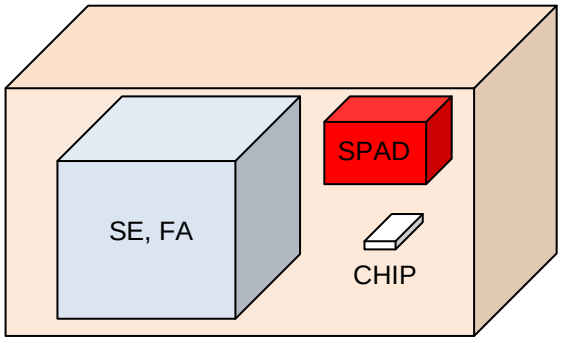

Figure 3: schematic view of the analytical device.
SE System electronics, FA fluidics actuator.

SPAD camera requires a re-design according to the specific space application and environment. SPAD dimensions are 82X50X60.

Chip design with integrated waveguides has to be carried out. It is feasible and already performed also for commercial instruments.

Electronics and actuation system development is considered as no-risk activity.

A technological demonstrator can be fully developed whitin 1-2 years.

8 CONCLUSION

Lab on a Chip technology is claimed to be a revolutionary technology for research in Life Sciences and biomedical application. Presently, integration of detection capabilities in LoC is an active field of research but still a technology capable of conquering the market is far to be available. Yet, at research level there is not a mature technology for full integration of optical detection in LoC.

Hybrid technology based on docking station and chip interface seems to be capable of overcoming technological limitations for a fully integrated LoC device.

The information in this document reports interesting features arising from optofluidics technologies. Especially due to the intrinsic sensibility of plasmonic and optical microresonators detectors. As a matter of fact, optofluidics is currently exploited in diagnostics and commercially available systems are already in the market.

Still, fluorescence is the eligible means for biological detection. Immune tests and nucleic acids amplification protocol (PCR, qPCR, NASBA) are worldwide performed by means of fluorescent labels.

The Q-SENSE PLC platform is a robust compromise: integration of optical components on the chip although limited to collection and excitation waveguide is proven to be effective. Moreover, detection is based on fluorescence measurements thus test sensitivity (e.g. LOD) is remarkable.

PLC has to be referred to as a candidare technology for space application. Nevertheless, hybrid integrated circuit based on optofluidics and standard technologies can be envisioned.

With respect to boundaries of integration in LoC, our study suggests that detectors are to be considered as an external components. Waveguides have to be considered as integrated components.

SPAD technology for single photon counting and thus extreme fluorescent detection is available. Thus, LoC with integrated optical components and biochemical assay can be coupled with an effective detection technology.

In view of development of LoC based bioanalyzer for space application a sensor capable of photon counting, as the SPAD one, will provide unmatched detection sensitivity.

So far the limiting factor in the attempt of performing biological detection in LoC relies on the paucity of tests that has been downscaled by laboratory standard protocols to microlitre volumes.

APPENDIX 1
Table of bioparameter considered of interest for space activities.

Bioparameters from RD05			
1	Creatinine	52	Anti diuretic hormone
2	Free tiroxine	53	Atrial natriuretic peptide/factor
3	Total tiroxine	54	Nitric oxide
4	Total triiodothyronine	55	Melatonin
5	Thyrotropin	56	Aldosterone
6	Type 1 procollagen terminal propeptide	57	Endothelin 1
7	Intact parathyroid hormone	58	Prostaglandins
8	1.25-(OH)2 vitamin D3	59	Urodilatin
9	Serum bone alkaline phosphatase	60	Lactic acid
10	Osteocalcin intact (or Meta / N-MID Osteocalcin)	61	Glucose
11	Ostecalcin fragment	62	Insulin
12	Urinary free deoxy-pyridinoline	63	Hemoglobin
13	Urinary pyridinoline	64	Hematocrit
14	Carboxy terminal telopeptide of type I collagen	65	Creatine kinase total
15	Insulin like growth factor 1	66	Creatine kinase MM
16	Total Ca	67	C-peptide
17	Total proteins	68	Myoglobin
18	Albumin	69	Total testosterone
19	H^+, pH	70	Creatinine
20	Cortisol	71	Angiotensin 2
21	Human growth hormone	72	Osmolality (mOsm/Kg)
22	Transforming growth factor beta 1	73	Somatostatin
23	Estradiol	74	Glucagon
24	Calcitonin	75	Free fatty acids
25	Osteopontin	76	Gastrin
26	Progesterone	77	Bilirubin
27	Interleukins (IL1-6, 11 13)	78	Gastric inhibitory peptide
28	Vitamin K	79	Pancreatic polypeptide
29	Proteoglycans	80	Secretin 1
30	Fluoro profile epicocconone	81	Prostaglandins E2
31	Osteoprotegerin	82	Adrenocorticotropin hormone
32	Receptor activator of nuclear factor (NF)-kB ligand	83	Alanine aminotransferase
33	Interferon gamma	84	Amylase
34	Macrophage colony-stimulating factor	85	Anion Gap
35	Transforming growth factor beta 1	86	Aspartate aminotransferase
36	Na	87	C-reactive protein
37	K	88	Ferritin
38	Cl	89	Free testosterone
39	HCO3	90	Free tryptophane
40	I P	91	5 Hydroxytryptophane
41	I Ca	92	Glutamine

42	Mg	93	Human chorionic gonadotropin
43	pCO2	94	Lactate dehydrogenase B
44	O2	95	Luteinizing hormone
45	Base excess	96	Follicle stimulating hormone
46	Epinephrine	97	Urea
47	Norepinephrine	98	Proteins
48	Dopamine	99	Bile salts
49	Blood urea nitrogen	100	Urobilinogen
50	Renin	101	Vanillyl mandelic acid
51	Brain natriuretic peptide		