Final Report Summary - PBSA (PHOTONIC BIOSENSOR FOR SPACE APPLICATION)

Executive Summary:
Life is considered as a planetary phenomenon, and, besides Earth, it might have evolved in other extraterrestrial bodies. The definition of the concept of Life is not straightforward but current exploration techniques are mostly related to Earth living organisms. The hypothesis currently used is based on the assumption that, under similar environmental conditions, organisms respond and adapt with similar strategies and molecular mechanisms. Therefore one of the most direct approaches to detect life is by searching for microorganisms or the molecular remains (biomarkers) they leave in the environment. The PBSA project addresses the search for molecular evidences of life by automating bio-affinity based methods, as the immunological detection or the specific recognition between complementary DNA sequences, in a direct label-free and near real-time biosensor device.

The PBSA goal is the implementation of Lab-on-a-chip (LoC) concepts for biomarker detection in space applications such as life detection missions or microbial monitoring in space installations. PBSA combines
advanced terrestrial technologies in the field of biosensors for the development of a novel and integrated solution ready to be tested for the space environment. This approach employs recent advances in immunosensing technology developed for planetary exploration with two main powerful technologies, photonic integrated circuits (PICs) and microfluidics, with promising features for the space domain. The use of PIC enables the implementation of highly integrated solutions for new LoC developments. Multiple detections can be integrated into a single photonic chip for multiple parallel analyses. This technology has shown to be very sensitive and improves protocol simplicity compared with conventional techniques that require enzymatic or fluorescent labelling steps. The PIC based solution allows direct measurement of the target molecules (analytes), savings time and complexity of the detection protocols with similar liabilities. Moreover, this feature is very suitable for remote sensing in space applications where savings in reagents are very valuable.

Partner’s skills are very well complemented in the project. The know-how in immunoassay chemistry for space applications is combined with the expertise in CMOS photonic integrated circuits and active microfluidics. Finally a specialist in space will do control electronics and integration.

Both photonic and microfluidic systems optimize critical parameters in space applications (volume and mass) enabling new opportunities. These benefits apply also to terrestrial market. The huge growth of the terrestrial biosensing field has been mainly focused to simple low cost devices. In this sense, the search of organic molecules of unambiguous biological origin is also related to ground disciplines such as biomedicine (human, animal), bioterrorism, or environmental or industrial processes monitoring. The underlying biological system and types of biomolecules is common in these fields and the same detection mechanisms can be employed.

Consequently, PBSA has generated a unique, light (1.2 kg) and compact (131x131x154 mm) prototype instrument for multiplex (up to 10 different analytes can be detected simultaneously) and label-free immunological detection. We have designed and built a unique microfluidic cartridge that contains all the reagents necessary for two consecutive analyses with a single, reusable, photonic integrated circuit and a small array with 12 micro-ring resonator probes.

Project Context and Objectives:
4.1.2.1. Scientific context
The PBSA project arises as a response to the demanding necessity of new affordable, light and “user friendly” biosensing devices in the context of space exploration. Two fields might demand PBSA: Astrobiology and human space exploration. The search for extra-terrestrial life is a hallmark in the human knowledge, and Astrobiology addresses it through the study of the origin, evolution, distribution and future of life in the universe. The interest in exploration of life on other planets or moons is not new. It started when it was discovered that comets contributed with water and organic materials to the primitive Earth. The NASA’s 1976 Viking missions to Mars were the first space missions with clear astrobiological objectives. The inconclusive results from the Vikings missions about the presence of organic material or any other life remains led to a period of scepticism and absent of planetary missions. New findings about the recalcitrant nature of microbial life, able to proliferate under extreme environmental conditions on Earth, together with the improvement in the sensitivity of the new instrumentations, prompted scientific community and, in particularly NASA, to support and provide funding to astrobiology by the middle 90s. On the other hand, the human space exploration demands new monitoring systems ether for habitat management or health checking.

Why to search for organic matter in the space: planetary and human exploration
The detection of organic matter from unequivocal biological origin is essential to evaluate the presence of extant or extinct life in a planetary body. Our hypothesis is based on the assumption that, under similar environmental conditions, organisms respond and adapt with similar strategies and molecular mechanisms. If we know those terrestrial analogue environments (at least in some key environmental parameters) to other identified on other planetary bodies, we characterize some of the life forms and the remains they leave in the medium, and develop systems for detecting them, then we could extrapolate this methodology for planetary exploration.

The most direct way to identify the presence of life, past or present, is through the detection of the molecules that compose it (molecular biomarkers) or the organic matter originated from large biological polymers. In the case of modern life, the biomarkers may be different polymers types more or less complex such as proteins, nucleic acid fragments (DNA, RNA), lipids, or biogenic polysaccharides (lipopolysaccharides, teichoic acids, etc). In the case of extinct life, the most relevant biomarkers are the products generated by transformation (diagenesis) of the different biomolecules from the cell, such as humic acids, hopanes, isopenoids, etc.

The detection of molecular biomarkers not only help to search for extraterrestrial Earth-like life, but it can also help in human exploration by habitat monitoring or checking different health parameters of the astronauts. For example, monitoring the air, surfaces or water reservoirs, or by checking different illnesses or microbial infections during long stay missions. In that sense, it is important to routinely check for potential microbial pathogen contamination in space stations or future planetary settlements. For these purposes fast and reliable sensor system are desirable.

Bioaffinity-based sensor such as the immunosensors can detect a wide range of sizes and molecular structures, from amino acids to whole cells, using relatively simple and robust protocols. The antibody microarray technology allows analyzing hundreds or even thousands of different types of molecules simultaneously.

The technology that we propose in PBSA has an important niche in the instrumentation used in the search for life, covering aspects that no other instrument has addressed so far: the ability to recognize and detect non-volatile complex molecular structures by label-free immunoasays.

4.1.2.2. Technological context

Up to date, the instruments devoted to organic detection in space missions have been based on the analysis of the volatile compounds released after sample heating or pyrolysis (Table 1) mainly due to the simplicity in sample preparation. This is the case for gas chromatograph-mass spectrometers (GCMS). Two classes of instrumentation have been used in the search for organics compounds and life on planetary exploration: the classical biology and chemistry instruments, e.g. labelled release (LR) and the gas chromatography / mass spectroscopy (GCMS) as it was used on Vikings landers in 1976 on Mars.

The LR experiment (Principal Investigator Gilbert Levin) tested for life by applying a nutrient solution tagged with Carbon 14 to a sample of Martian soil.

While best known for detecting water ice near the Northern Polar Cap, Phoenix also detected perchlorates. A critical step with a GCMS is to heat the sample to make it outgas. Ideally this is done in an oxidant-free oven to prevent spurious heat reactions. But because perchlorates are an oxidant in the presence of organics, they form dichloromethane when heated with organics. And indeed, Curiosity’s GCMS has recently detected still more dichloromethane in 2012 in the first analysis of the SAM instrument. Only after using chemical derivatizing agents SAM have detected the most complex organic matter found so far on Mars: Benzene.
To search for more complex and polymeric non-volatile biomarkers we proposed several years ago the bioaffinity-based method of immunological detection (Parro et al., 2005; 2008, 2011). Antibodies are big (150 kDa, about 7-9 nm diameter particles) and relatively robust molecules capable to specifically discriminate between enantiomeric compounds with high sensitivity. The most reliable and sensitive methods rely on the fluorescent or enzymatic labelling of antibodies. Although highly effective, these methods need laborious preparatory processes, liquid handling and incubation times ranging from 30 to 120 min, apart of the signal detection system.

PBSA project goes a step forward by simplifying the analytical process through label-free photonic detection in a near real-time reading system. PBSA is focused on the implementation of a Lab-on-a-chip (LoC) concept where advanced terrestrial technologies in the field of biosensors (photons, microfluidics and electronics) are combined with the last advances in biochemical functionalization of surfaces for the development of a novel solution for space applications.

The use of photonic integrated circuits (PIC) enables the implementation of highly integrated solutions for the development of new LoC. This technology has shown to be very sensitive and also has the advantage of being a label-free and near real-time detection technique. Moreover the implementation of microfluidics cartridge at the device allows a low volume consume for the reagents. Furthermore, a very important feature of the project is the possibility of a multiple detection immunoassay.

4.1.2.3. Objective
The PBSA goal is to develop a compact photonic-based biosensor prototype for the simultaneous detection of several biomarkers or microbes and to demonstrate its feasibility in the context of space exploration. Because the target analytes to be detected are of similar nature as those targets in many terrestrial fields (biomedicine, veterinary, environmental monitoring), PBSA is also relevant for these ground applications.

The particular PBSA objectives are
a. To bring nanophotonic technology to space environment.
b. Improve space sensing capabilities with photonic solutions
c. Develop a nanophotonic array of sensors capable to operate in space environment (radiation).

To achieve the objectives, we have established the next work packages:
• WP1: Conception of the system: Study of Space application requirements
• WP2: Development of the sensing probes and immunoassays
• WP3: Development of photonic transducers
• WP4: Development of microfluidic structures
• WP5: Integration and Electronics
• WP6: Validation of the functionality
• WP7: Dissemination and exploitation plan
• WP8 and 9: Project management and Technical and Scientific coordination

Project Results:
See attached document: PBSA_final_report_en_V1

4.1.3. Main S&T results and foregrounds
4.1.3.1. WP1: Conception of the system: Study of Space application requirements
Objectives
Objectives

- Study of space mission requirements and their impact on the device
- Definition of the target application: determination of target molecules to be sensed.
- Building block breakdown of the sensing device and main action to adapt them to the space application.
- Definition of the interrelation between building blocks.
- Draw of the main action lines in each of the main parts of the system for WP2, WP3, WP4 and WP5.
- Definition of the objectives and protocols for the validation experiments in performance and in environmental conditions for WP6.

Results

- State of the art of sensing solutions for space and terrestrial applications has been provided in order to put PBSA project into its context (Fig. 1). The search for organics and molecular biomarkers is a main goal in planetary exploration. Finding molecules of unequivocal biological origin may constitute direct evidence of the actual or extinct life in other planetary bodies. In order to search for those molecules of unequivocal biological origin, one option is to search for the most characteristic molecules of life as we know it: the biological polymers such as proteins (polymers of aminoacids), DNA and RNA (polynucleic acids), polysaccharides (sugar polymers), or unique and sophisticated molecules produced by enzymatic reactions, such as steroid-like compounds, antibiotics, etc. (Fig. 2). In addition, it is necessary to understand how biological molecules are transformed with time and the different diagenetic processes that can operate over them. The main molecular biomarkers from polymers to more simple molecules obtained after diagenesis was reviewed in PBSA deliverable D1.1.

Figure 1. PBSA potential application and targets

- The required validation test and protocols for PBSA has been initially defined in deliverable 1.4. Therefore, they were used as the base reference for the validation test to be performed during WP6.

Figure 2. The main biological polymers constituents of life are targets for PBSA (left) and fossil molecular biomarkers (hopanes), humic acids, bacterial pigments and polycarboxylic acids from oxidized organic matter are potential targets (right).

- Architectural PBSA design (Fig. 3) has been provided, including building block decomposition and interrelation. The different electrical, fluidic and optical external and internal interfaces were defined. They were used as a base for the WP5 activities.

Figure 3. PBSA system concept block diagram

- Main block interdependencies and critical parameters were defined (e.g.: tuning range of the laser with hope mode free, size of the photonic integrated circuit, packaging, maximum size of particle in the microfluidic system and functionalization methods).
- We constructed three developmental models, proto-lab 0, 1 and 2, to demonstrate PBSA concept and to test and improve the different procedures and components.

4.1.3.2. WP2: Development of the sensing probes and immunoassays

Objectives

- Selection of target analytes and the biorecognition elements (bioreceptor molecules)
- Functionalization of PIC transducers: Immobilization of bioreceptor molecules to the micro-ring resonators. This requires the design of the immobilization protocols and printing strategies.
To achieve the objectives we have done multiple tasks. In biology many activities have to be repeated multiple times because biological and biochemical reagents need to be replaced in each experiment. Therefore, we have done tasks such as fabrication of multiple PIC chips, chemical activation, functionalization with antibodies (using the printing robot), immunoassay tests and photonic measurements with the different opto-microfluidic set ups (proto-labs 0 to 2). Printing of conventional antibody microarrays onto glass slides has also been done as routine check of the antibody performance by fluorescent immunoassay.

Results

A multiplex label-free immunoassay for PBSA

Multiplex functionalization following epichlorhydrin method (Fig. 4) was applied to 12 microrings PICs (Fig. 5) to be implemented into PBSA prototype. Our strategy is to functionalize several rings with the same antibody in order to have redundancy and the appropriate controls to measure the reproducibility of the assay. For the purpose of this project, the most critical aspect is to obtain consistent measurements. From all the 300 antibodies shown in Table 8, D1.1 we have just selected a very few of them (Table 3) but highly representative of the potential applications of PBSA: antibodies to the universal bacterial protein GroEL, antibodies to spores (resistant cellular forms) from the widely distributed in nature bacterium Bacillus subtilis, or an antibody to a protein from a viral pathogen affecting cattle. In the last case, it was a part of collaboration with the RAPIDIA-FIELD FP7 project and as one of the potential applications in the veterinary diagnostics, we added biomarkers for viral diseases.

Table 2. Antibodies used for multiplex functionalization of the PBSA development PIC chips

<table>
<thead>
<tr>
<th>Ab Name</th>
<th>Type</th>
<th>Antigen</th>
<th>Justification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-GroEL</td>
<td>Poyclonal</td>
<td>GroEL (chaperone 60)</td>
<td>Universal bacterial protein</td>
</tr>
<tr>
<td>IVH1C1</td>
<td>Poyclonal</td>
<td>Bacillus subtilis spores</td>
<td>Example for detecting resistant bacterial cells</td>
</tr>
<tr>
<td>Anti-Cys</td>
<td>Poyclonal</td>
<td>Cysteine-BSA conjugated</td>
<td>Example of amino-acids</td>
</tr>
<tr>
<td>Anti-GlnB1492</td>
<td>Poyclonal</td>
<td>17 aa peptide of protein GlnB (L. ferrooxidans extremophile from Río Tinto)</td>
<td>A small peptide</td>
</tr>
<tr>
<td>2C4</td>
<td>Monoclonal</td>
<td>VP7 protein from BTV Virus capsid antigen</td>
<td>Bovine pathogen (Bluetongue virus) from other FP7 project collaboration (RAPIDIA-FIELD)</td>
</tr>
</tbody>
</table>

These antibodies were printed on the final PBSA PIC chip and assayed with proto-lab 2 set up. We successfully performed multiplex label-free detection of GroEL, spores and VP7 protein by using the same chip (Fig. 6). The changes in the photonic resonance were calculated by subtracting the resonance displacement of the test ring minus the value obtained in other rings functionalized with other antibodies or just with BSA (Bovine Serum Albumin). Table 2 shows the values obtained in one of the experiments.

Figure 4. Chemical activation of the micro-rings for covalente binding of proteins and antibodies.

In order to increase the photonic signal and, consequently, the sensitivity, we applied a signal enhancement strategy by adding a secondary antibody and a nanoparticle-based system (Fig. 6, D). Because antibodies can be considered as a kind of nanoparticles (7-9 nm in diameter), after the addition of a secondary antibody the photonic signal increased considerably (Fig. 6).
Figure 6. Multiplex label-free immunoassay and enhanced procedures for PBSA. Resonance displacement measurements for the detection of VP7 viral antigen (A), GroEL protein (B) and Bacillus subtilis spores (C). In (D) it is shown the interpretation of the immunoassay following the different theoretical parts of photonic assay: Running Buffer (RB), Antigen or analyte addition, RB, antibody addition for enhanced sandwich, and nanoparticle addition for further enhancement. The figures are more extensively explained in D2.4.

Table 3. Photonic measurements in multiplex label-free and enhanced protocols

<table>
<thead>
<tr>
<th>Sample injected</th>
<th>Immunoassay type</th>
<th>Photodetector minus Reference Signal (pm)</th>
<th>Total Signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>VP7 50 ng/ml</td>
<td>Label-free</td>
<td>PH1-PH3</td>
<td>2.0 2.8</td>
</tr>
<tr>
<td>Ab 2D7 Sandwich</td>
<td>PH1-PH3</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>GroEL 0.5 ug/ml</td>
<td>Label-free</td>
<td>PH2-PH1</td>
<td>34.0 82.0</td>
</tr>
<tr>
<td>Ab Anti-GroEL biotinilated Sandwich</td>
<td>PH2-PH1</td>
<td>48.0</td>
<td></td>
</tr>
<tr>
<td>GroEL 0.5 ug/ml</td>
<td>Label-free</td>
<td>PH2-PH3</td>
<td>29.0 67.0</td>
</tr>
<tr>
<td>Ab Anti-GroEL biotinilated Sandwich</td>
<td>PH2-PH3</td>
<td>38.0</td>
<td></td>
</tr>
<tr>
<td>Spores (B.subtilis)</td>
<td>Label-free</td>
<td>PH3-PH2</td>
<td>1.1 14.1</td>
</tr>
<tr>
<td>Ab IVH1C1 Sandwich</td>
<td>PH3-PH2</td>
<td>13.0</td>
<td></td>
</tr>
<tr>
<td>Spores (B.subtilis)</td>
<td>Label-free</td>
<td>PH3-PH1</td>
<td>1.4 16.0</td>
</tr>
<tr>
<td>Ab IVH1C1 Sandwich</td>
<td>PH3-PH1</td>
<td>12.0</td>
<td></td>
</tr>
<tr>
<td>Spores (B.subtilis)</td>
<td>Label-free</td>
<td>PH3-PH4</td>
<td>0.7 6.6</td>
</tr>
<tr>
<td>Ab IVH1C1 Sandwich</td>
<td>PH3-PH4</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>GroEL 0.5 ug/ml</td>
<td>Label-free</td>
<td>PH2-PH1</td>
<td>5.0 73.0</td>
</tr>
<tr>
<td>Ab Anti-GroEL biotinilated Sandwich</td>
<td>PH2-PH1</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>Stv-nanoparticles</td>
<td>Nanoparticles</td>
<td>PH2-PH1</td>
<td>60.0</td>
</tr>
</tbody>
</table>

Our results showed that it is feasible the multiplex immunoassay (that is simultaneous detection of several biomarkers or microbial strains) with a single chip. The enhancing procedures can be run in the same chip, and clearly increase the photonic signal (Figure 1 and Table 2).

The limit of detection of PBSA photonic immunoassay

We also determined the limit of detection for some biomarkers by label-free assay, as it was the case of GroEL protein (Figs. 7 and 8), which resulted in a value of 10 ng/ml. This limit of detection is in agreement with the the one obtained by fluorescent sandwich immunoassay (2-5 ng/ml).

Figure 7. Detection limit of the PBSA PIC system for detecting GroEL protein. (blue) the result of subtracting the photonic measurement in photodetector 2 (Fd2) minus the reference photodetector 1 (Fd1), and (red) after subtracting Fd3.

GroEL (ng mL-1) or ppb Anti-GroEL (5 μg mL-1)

Figure 8. Limit of detection of a photonic label-free immunoassay for GroEL protein

Reusability of PBSA PIC chip

We also examined the reusability of the chip by performing up to ten cycles of label-free assay + regeneration with 20 mM NaOH (Fig. 3). Although the system can be improved, after 10 cycles we still...
Regeneration with 20 mM NaOH (Fig. 3). Although the system can be improved, after 10 cycles we still detected a similar photonic measurement as with the first cycle.

Regeneration Cycle

Figure 9. Regeneration of PBSA PIC immunoassay. Photonic label-free immunoassay for detecting GroEL protein by using a single re-generable chip. The label-free assay (blue bars) was done 8 times after regeneration with 20 mM NaOH. In some of the cycles, the sandwich-type enhanced protocol was applied (red bars). The values are the average of the measurements obtained with two different rings functionalized with Anti-GroEL antibody. Error bars correspond to the standard deviation. In cycles 2 and 9 only regeneration was applied.

The reusability of the chip is feasible and it would save time and money when performing the analysis of several samples in short periods of time. However, to do that it is necessary to re-design the microfluidic cartridge so that the pumping system can be much more accurately controlled.

Conclusion and Perspective

We have developed a sensitive multiplex label-free immunoassay for PIC

Two procedures for enhancing the photonic signals have been demonstrated

PIC for PBSA will be re-generable for at least ten times

Although there are still several parameters to be optimized, we have demonstrated the performance of PIC chips for label-free, near real-time, and simultaneous detection of several biomarkers, regardless their nature. The final performance of the measurements depends of multiple factors, such as: The quality of each pair antigen-antibody or any other bioreceptor pair molecules, the functionalization efficiency, or the photonic efficiency of the chip, among others.

4.1.3.3. WP3: Development of photonic transducers

Objectives

• Assembly PIC transducer
• Design of Light interrogation system and coupling
• Optical characterisation of PIC transducers

The fabrication of the photonic structures such as the ring resonators and waveguides involves nanofabrication processes by e-Beam lithography and photolithography. Those processes are followed by dry etching (plasma etching) to transfer the pattern onto the silicon nitride surface.

Once the PICs are fabricated and characterized, the next step is the attachment of the input and output ports. For the input-port a Fiber-array (FVA) is attached to transmit the laser light signal into the PIC. As output-port, a set of Photodetectors (PDA) is bound to the PIC to convert the photonic signal into an electrical domain (Fig. 10).

The illumination control and monitoring system takes care about the light generation system (laser sweep needed for exciting the transducer) and the measurements of the wavelengths variations produced because of the sensing interaction.
Because of the sensing interaction.

Results

Assembly of a compact ad integrated PIC transducer

For the characterization of the PIC transducer, FVA attachment and PDA need to be tested. FVA characterization was done to assess the correct alignment. For test the performance of the FVA were used a broadband ASE source centred in 1550nm and by means of GC1 and GC3 were maximized the power coupled to ensure that the GC2 was aligned with the FVA.

Table 4. Table Alignment losses at the GC input (using GC1, GC3) from 3 measured chips

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>4:6_4</td>
<td>-18.4</td>
<td>1.3</td>
<td>1.4</td>
<td>-19.8</td>
</tr>
<tr>
<td>6:5_4</td>
<td>-16.5</td>
<td>1.2</td>
<td>1.4</td>
<td>-17.9</td>
</tr>
<tr>
<td>6:1_2</td>
<td>-19.6</td>
<td>1.1</td>
<td>1.2</td>
<td>-20.8</td>
</tr>
</tbody>
</table>

The losses are higher than expected for only the inter-phase of 2 GC due to the source used for this test is an ASE source and the power coupler to the fibers is a bit lower than in the case of a connectorized laser monochromatic as is the case of the laboratory test.

The last results obtained from the laboratory tests, were using the PBSA Electronic Control Unit (ECU) made by EVOLEO partner and using the tunable laser EM650. Within these tests we made an electronic sweep to the laser by controlling the temperature input to tune the wavelength and obtaining the response of the system to each wavelength. The Fig. 11 shows the obtained response, which corresponds with the resonance of the ring resonator previously measured. It can be observed the deep expressed in voltage from the TIA board, due to the PD converts the optical power from the PIC circuit into a current and then the TIA convert this current to voltages. Adjusting each amplifier to the concrete RR can do some improvements of the system. In this way, it is possible to get the best dynamic range that can provide the TIA board (max range ~0-5 V).

Figure 11. Electrical response of the PD using the complete system.

Optical characterisation of PIC transducers

The main procedures for PIC characterization are physical and optical characterization.

- The physical characterization relates to the physical measurement of the fabricated structures. For that purpose, SEM (Scanning Electron Microscope) and AFM (Atomic Force microscope) images are obtained in order to check specifications in terms of fabricated dimensions with respect to the original design. Additionally, interferometric techniques are employed in the fabrication process in order to verify layer’s thickness.

- In the photonic field, there are some tricky issues as the coupling of light inside the chip, which makes necessary the use of a laboratory characterization set-up. From this set-up, the optical pathway has been employed for the characterization of the fabricated chips. It basically consists of a tuneable laser source at one end of the system, and a power meter at the other. The wavelength of the laser is shifted in time to obtain the spectral response of the device. A positioning system with 6-freedom axis has been used to achieve the most accurate alignment of the fiber, having in mind that the alignment of fiber array is necessary to measure structures in parallel. A polarization controller is used in order to maximize the power on the chips since the structures used to coupling light inside the chips are polarization dependents.
power on the chips since the structures used to coupling light inside the chips are polarization dependents.

Table 5. Specs of the optical characterization of the implemented set-up

Conclusions

Fabrication processes have been developed and optimized for silicon nitride technology and the PIC transducer for PBSA has been assembled

The implemented light interrogation and readout system is working well

The PBSA photonic integrated chip (PIC) was optically and electrically characterized and the obtained results were well in line with the simulations

4.1.3.4. WP4: Development of microfluidic structures

Objectives

- Design and production of PBSA fluidic cartridge.
- Developing, preparing and testing cartridge technology for implementing photonic-based sensor technology and meeting the requirements of the sensor concept.
- Design of pump control.

Results

We have designed and produced a unique microfluidic cartridge for PBSA (Fig. 12). The cartridge contains several reservoirs for chemicals and reagents sufficient for full analysis of 2 samples, including enhanced protocols with secondary antibodies and nanoparticles. For those assays were no enhancement of the signal is needed, the extra reservoirs can be filled in with other reagents and used for an extra analysis.

Figure 12: Picture of the cartridge filled with inks representing reagents and labelled essential areas.

Through the cartridge assembly process it was possible to reproducibly build up cartridge batches to be used by the project partners for system level validation and biochemical experiments (Fig. 13).

Figure 13. Picture of a batch of 20 cartridges to be send to project partners for system level validation and biochemical experiments

Pump Control Design

The liquids in the reservoirs of the microfluidic cartridge are pumped through a electrochemical pumping system that needs special control. An OEM pump control was developed based on devices of the past. This allowed a fast delivery of a board controlled by the PBSA system, which is able to drive the pumps on its own (see D4.2).

For a fully integrated system and for the space conformity, the consortium agreed to develop a software component to be integrated into the PBSA environment (Fig. 14). The pump control software component is a library ("Pump Control DLL"), which can be integrated into the software environment ("Application Control") so far developed for PBSA. The “Application Control” transmits status data for the pump to be controlled:
- "Volume": Status of the pump, namely volume already pumped.
• “Volume”: Status of the pump, namely volume already pumped
• “Pump”: Number of the pump to be controlled
• “Flowrate”: desired flowrate

The “Pump Control DLL” calculates a current, which is than set within the “Drive Electronics”.

Figure 14: PBSA system architecture with focus on the communication of the PBSA system with the pump control software component and the driver electronics, which finally drive the pumps

The PBSA system can drive the pump to achieve different flow velocities of the reagents within the sensor chamber. A typical flow velocity variation range is within 0.01µl/s and 2µl/s. The availability of high flow velocities is highly dependent on the reservoir geometry. For the PBSA specialized cartridge flow velocities up to 0.9µl/s can be controlled without any additional considerations.

Conclusions

A unique microfluidic cartridge with pre-loaded reagents and electrochemical pumping system has been designed, built, and tested for performing 2 immunological assays in PBSA

4.1.3.5. WP5: Integration and Electronics

Objectives
• Final concept definition including thermal considerations.
• Laser control board.
• Photodetector Signal Conditioning board.
• Electronic Control System board.
• PSU and the power distribution unit.
• Final integration and verification tests of the PBSA prototype

Results

As a result of this WP fully final design was done for each individual component and the whole assembly was implemented. After final design of all the components, procurements, and construction of all PBSA parts, the final integration was achieved to obtain the PBSA prototype instrument (Fig. 15). We carried out the verification tests: power supply, voltage to all components, microfluidic control, laser control, photonic measurements, etc.

Figure 15. PBSA prototype. Integration of the final and operative PBSA prototype

Figure 16. Checking the PBSA software and user’s interface and record of the first photonic measurement

Conclusions

We have built and verified the main functionalities of PBSA prototype

4.1.3.6. WP6: Validation of the functionality

Objectives
• Implementation of detection protocols.
• Performance validation of the PIC based label-free detection system and microfluidics. Comparison with other techniques.
• Environmental tests of the developments of the PBSA project
Environmental tests of the developments of the PBSA project.

Results

PBSA protocol

We have designed and tested a label-free immunoassay for PBSA prototype. Once produced the microfluidic cartridge and the functionalized PIC as described in the corresponding deliverables, and based on the multiple assays performed with the different proto-labs versions, we described a detailed protocol (D6.1) consisting of the next steps:

Table xx. PBSA running protocol
Summary of PBSA Running protocol for sample analysis

a. Fill in the cartridge reservoirs with the corresponding reagents and solutions
b. Fill in the sample reservoirs with the corresponding samples 1 and 2
c. Fix the PIC chip to the cartridge and then to PBSA machine
d. Open PBSA software interface
e. Selection of the pre-stored running program and press run, or define a new running procedure (Table 2)

Table xx. Step by step programming PBSA running protocol

Programming PBSA running protocol

1. Optical setting (laser, signal recording, e.g. Fig. 15)
2. Blocking buffer (vol, velocity, t)
3. Running buffer (vol, velocity, t)
4. Sample 1 pumping into the microfluidic cell (vol, velocity, t)
5. Running buffer (RB) (vol, velocity, t)
6. Secondary antibody (vol, velocity, t) if needed
7. Running buffer (RB) (vol, velocity, t)
8. Regeneration buffer (vol, velocity, time)
9. Sample 2 pumping into the microfluidic cell (vol, velocity, t)
10. Running buffer (vol, velocity, t)
11. Secondary antibody (vol, velocity, t) if needed
12. Running buffer (RB) (vol, velocity, t)
13. Regeneration buffer (vol, velocity, time)
14. End of run

The microfluidic cartridge was designed to run 2 samples and with several reservoirs to store different reagents. Multiple reservoirs give versatility and allow the user designing multiple protocols as a function of the type of immunoassay used: direct label-free, sandwich, further enhanced with nanoparticles, or even enzymatic enhancement (not tested yet).

Tests under space relevant conditions

Any new instrumentation or device claiming for space applications needs to demonstrate its good performance under critical space environmental parameters. Radiation is one of the most critical, particularly the high-energy proton radiation and the less energetic but highly penetrating and highly abundant gamma radiation. Many instruments have been flown with electronics, optical CCDs, software, mechanical components, etc. However up to now none have used SiN waveguides-based photonic chips or electrochemical pumped microfluidics, and much less biochemical components as proteins and
of electrochemical pumped microfluidics, and much less biochemical components as proteins and antibodies.

Consequently, the photonic chips, the microfluidic cartridges, and the antibodies used in PBSA have been tested under space relevant radiation conditions:

i) more than twice the high energy proton radiation that a chip would receive in a 2 years mission to Mars, and

ii) more than 1000 times the gamma radiation in the same mission conditions.

No significant alteration in the performance of any of the three components was observed (Figs. 17-19).

Figure 17. Effect of gamma radiation on the microfluidic cartridge. This amount of radiation (15 krads) does not affect the cartridges behaviour. The gamma radiated cartridges with 15krd results (black and blue) in comparison to reference failure tube. Similar effect was observed for proton radiation (not shown here. See D6.3)

Figure 18. Effect of gamma radiation on PIC. Similar behaviour of 3 rings before (left) and after irradiation (right). Similar effect was observed for proton radiation (not shown here. See D6.3

Figure 19. Effect of high-energy proton and gamma radiation on immobilized antibodies. Antibodies anti-GroEL and anti-Bsubtilis spores (IVH1C1) were printed onto microscope slides and then irradiated with high energy protons and gamma radiation (blue, non-irradiated; red irradiated with 15 krad of protons, more than twice the radiation that antibodies could receive in a 2 years mission to Mars. The printed antibodies were used to measure different concentrations of GroEL. The plots represent the relative fluorescence. The behaviour of the irradiated (red) is similar, even slightly better, that the non-irradiated ones (blue) for the GroEL detection. For the anti-spores it is similar, although one of the spot is out of the curve

All the electrical and mechanical components selected and acquired for PBSA are space qualified by the provider, so we do not consider necessary to subject them to space environmental tests. The electrochemical pumping system and valves that retain liquids in the cartridge reservoirs need to be qualified under vacuum. We consider that valve qualification for space is out of the scope of the project and it would need extra funding.

Conclusions
We have developed and validated a versatile protocol for label-free near real-time detection of proteins and microbial cells in PBSA prototype

No effects on the photonic chip nor on the microfluidic cartridge was observed after high energy (protons) and gamma radiation exposure

PBSA is a prototype photonic based biosensor instrument for multiplex molecular biomarker or microbial cell detector

Potential Impact:
4.1.4. Potential impact
Final results

• The development of several activation + functionalization protocols for SiN micro-ring resonators
• A printing procedure for multiplex functionalization of PIC chips
• A multiplex label-free immunoassay protocol for PICs
• A unique microfluidic cartridge for performing the consecutive analysis of two samples by PIC and using enhancing signal procedures.
• A unique integrated PIC design for multiplex photonic detection
• And, finally, a unique prototype compact instrument for label-free near real-time multiplex immunological photonic detection.

Potential impacts

Although still depuration and refining improvement have to be done, PBSA can be a source of innovation that may bring new opportunities in the space and security applications. From PBSA device starting point, a list of high innovative products in high demanding markets like the ones mentioned below can be developed:

• Medicine and Veterinary: in situ and near real time assays. Our results with the antibodies to viral protein VP7 from Bluetongue virus demonstrate its utility in veterinary field. In fact, this antigen-antibody material was obtained as collaboration with another FP7 project: RAPIDIA-FIELD, devoted to the development of quick detection methods for animal pathogen in the field (farms) or small field lab. PBSA could be an excellent candidate.
• Bioterrorism and NBQ for quick and real time pathogen detection.
• Environmental monitoring application: pollution in the seas and lakes and rivers.
• Industrial applications where in-line monitoring capabilities are required inside the process.
• In the farm fisheries, where it is critical to detect the presence of pathogens or the proliferation of algae or the presence of certain toxins, the use of a continuous monitoring system would be of great utility to the industries of the sector.

Project public website: www.pbsa-fp7.eu

List of Websites:
PBSA web site: http://www.pbsa-fp7.eu

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