BIOSENSING TECHNOLOGIES FOR SPACE APPLICATIONS

Gianni Pezzotti, PhD[1,2*]; Juan Bernado Cano, PhD(c)[3]; Katia Buonasera PhD[2]; Maria Teresa Giardi, PhD[2]

[1]Istituto di cristallografia-Consiglio Nazionale delle Ricerche Via.Salaria Km 29,3-00015 Monterotondo(Rome)
[2]Biosensor srl - Via degli Olmetti 44 -00060 Formello (Rome) Italy
[3]Dep. Electronic Engineering - University of Rome "Tor Vergata" Via della ricerca scientifica 00133 Italy

**Corresponding at the author* Ph.D. in Sensor Engineering; Researcher IC-CNR[1] and Project engineer of biosensor[2] e-mail: gianni.pezzotti@mlib.ic.cnr.it

ABSTRACT

The main studies performed in space deal with the survival of the astronauts during flights of long duration. One of the most investigated issues is the continuous attack of the cosmic ray. In order to assess the effect of the cosmic ray, two instruments hosting various sensing elements have been designed and constructed: Photo II and Night Vision. The first is an optical sensor which flew on board Foton-M2 and Foton-M3 missions of the European Space Agency; the second is an instrument that maintains alive different types of biomediators. The second instrument, called Night Vision, maintained alive algal microorganisms containing eyespots and macular pigment similar to human retina, with the purpose to predict the effect of space radiation on the astronauts' eye, in order to obtain results applicable to future nutrition programs in space.

Keywords: *Space instrumentation, biomediator, biosensors, ionizing radiation, C. reinhardtii, eyespots, retina.*

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TECNOLOGIAS DE BIOSENSADO PARA APLICACIONES ESPACIALES

RESUMEN

Los principales estudios realizados en el espacio hacen referencia a la supervivencia de los astronautas durante los vuelos de larga duración. Uno de los temas más investigados es el ataque continuo de los rayos cósmicos. Con el fin de evaluar el efecto de los rayos cósmicos, dos instrumentos con la inserción de diversos elementos sensores han sido diseñados y construidos: Foto II y Night Vision. El primero es un sensor óptico que voló a bordo de Foton-M2 y Foton-M3, misiones de la Agencia Espacial Europea (ESA), y el segundo es un instrumento que mantiene vivo los diferentes tipos de biomediadores. El segundo instrumento, llamado Night Vision, mantiene en vida microalgas que contienen manchas oculares y pigmentos maculares de forma similar a la retina humana, con el propósito de predecir el efecto de la radiación espacial en los ojos de los astronautas, y generar resultados aplicables a los programas de nutrición futuras en el espacio.

Palabras clave: *instrumentacion espacial, biomediador, biosensores, i radiacion ionizante, C. reinhardtii, eyespots, retina*

1. INTRODUCTION

Space is an inhomogeneous and dynamic environment strongly influenced by solar activity and characterized by radiation of a wide range of energies, types and particle's fluxes, which are potentially dangerous to all living organisms. Radiation affects astronauts, crew, and photosynthetic organisms all in a negative way.

Exploratory missions as well as long-term permanence on the International Space Station highlighted the necessity to develop technologies aiming to the protection from cosmic rays; for this reason many experiments are being performed in space over the last years, in order to boost this sector. Such experiments have been carried out on board vehicles going to space for defined periods, and had the purpose of assessing the effects of cosmic injuries on living organisms.

Up to now the platforms that have been mostly utilized for going to space are: the Soyuz with the Foton compartment in the facilities of the Biopan (URSS), that are described in the figure 1 for the experiment Photo II, and more recently the shuttle program (NASA Atlantis and Endeavour shuttle) which arrived to the final era of the program in 2011. Other rockets exist as Arianne program of ESA, with the last vehicles mini shuttle Hermes and rockets Arianne 5, in phase of experimentation.

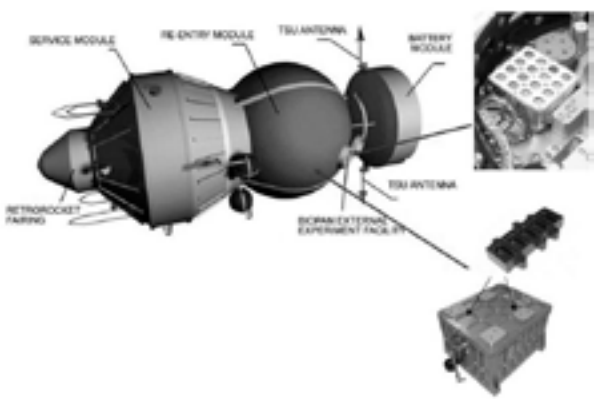

Fig. 1 Foton Biopan ESA Facilites for scientific experiments brought to space by Soyuz: Photo I was settled outside the foton spacecraft in the facility Biopan; Photo II multi-sensor was settled inside the Foton

Two important experiments have been performed on board Soyuz and Endeavour Shuttle The first, carried on by Photo II device, aimed to detect the effect of radiation on photosynthetic oxygenic microorganisms; the second, performed by Night Vision device, had the purpose of assessing the effect of cosmic rays on a structure present inside *Chlamydomonas. Reinhardtii* algae and mimicking human retina.

Photo II is a device that has been designed and developed within the framework of the Photo Project, as part of the Moma project "From Molecules to Man: Space Research Applied to the improvement of the Quality of Life of the Aging Population on Earth" funded by European Space and Italian Space Agencies [1].

On space flights Photo II measured the chlorophyll fluorescence induction curve in photosynthetic organisms, recorded and stored the data in a flash memory and provided the living conditions essential for the survival of the biological samples, by providing day/night cycles produced by white light emitting diodes (LEDs). Photo II used electronic components specifically designed to withstand Space conditions.
Night Vision has been design to host mutants of algae for the experiment titled "Eyespots and Macular Pigments Extracted from Algal Organisms Immobilized in Organic Matrix with the purpose to Protect Astronaut's Retina".

In one case, that of Photo II, the experiments performed in space stimulated the idea of taking advantage from the device designed for carrying on similar measurements on Earth.

As a result of Space-Earth technological transfer of Photo II, derived fluorescence based biosensors can be developed for application in environmental monitoring (e.g. water pollutants, quality control of drinking water), agriculture and industrial process control.

A biosensor is an apparatus that can detect a biochemical variable using a biological component (tissues, cells, enzymes etc.) interfaced with an electronic transducer. It produces an electrical signal that is easy to process that corresponds to the variable being analyzed. The biosensor is characterized by the sensitivity and selectivity of the response of its biological components and by the fact that it is economical, easy to use, of miniature size and versatile [2, 3, 4].

Biosensors have emerged as a promising technology especially in applications where real-time monitoring is required. This technology offers several advantages, since biosensors can be easily used both in laboratory and field applications [3].

Fluorescence biosensors allow simultaneous and multiparametric analyses to be performed, combining the three basic mechanisms of biological recognition: biocatalytic, bioaffinity and cell-based metabolic systems. In particular, among the sensing elements there are photosynthetic microorganisms, part of those, DNA, enzymes, binding-proteins etc. for the detection of several chemical species such as environmental pollution i.e. global toxicity, pesticides, pathogens and heavy metals [3, 5].

The experiment Night Vision is also described together with explanation of the importance of using biological organisms in space as models to achieve information which can be transferred on humans. Finally an illustration of Photo II technological transfer is given as the description of a series of biosensors designed and developed for application on the Earth [5, 6, 7, 8].

2. PHOTO II

Photo II was one of the experiments that composed the scientific payload of the satellites Foton M2 and M3 launched from the Cosmodrome of Baikonour (Kazakhstan), in 2005 and 2007 (see figure 2, showing the Soyuz before and after the flight). The Photo project investigated the possibility of using oxygenic photosynthetic microorganisms on long-term Space flights as a source of food, oxygen and nutraceutical compounds. The goal pursued was to assess the effects of the Space environment on various mutated microorganisms in order to select resistant-tolerant strains and determine the production of compounds with anti-oxidant properties resistant to Space ionizing radiation. Microgravity and ionizing radiation, which can influence the viability and performance of these organisms, are the critical points to resolve in utilizing plants or algae-based life supporting systems.

During the missions, Photo II monitored automatically the photosynthetic activity of several *Chlamydomonas reinhardtii* strains, unicellular green algae, carrying mutations in the D1 protein of Photosystem II [9, 10].

The D1 protein is a subunit involved in the formation of the core complex of PSII and it has a fundamental role in the photosynthetic process. Space ionizing radiation, various stress conditions and the absence of gravity can damage the D1 protein. Testing the radio resistance of D1-protein-mutants allows the amino acidic substitutions that are able to improve the tolerance of the microorganisms to the Space environment to be identified [9]. Microalgae are particularly suited as a regenerative-life supporting system as they have a low sensitivity to microgravity, a short life cycle, are easy to cultivate in photobioreactors and have high biomass productivity. They are also a rich source of secondary metabolites with anti-oxidant properties to provide a nutraceutical anti-oxidant-enriched biomass as a dietary supplement for the crew of spacecrafts. Some organic pigments play a key role in protecting photosynthesis under stress conditions; particularly xanthophylls that are oxygen-containing carotenoids, i.e. zeaxanthin, antheraxanthin, violaxanthin, involved in the photo protection of the photosynthetic apparatus. When the photosynthetic organisms are illuminated by a strong light, which exceeds their capacity for photosynthesis, the excess energy can be harmful for the photosynthetic apparatus. Under these conditions, the non-dissipating pigment violaxanthin is rapidly converted, via the intermediate antheraxanthin, to zeaxanthin, that has high photoprotective properties, dissipating the energy in excess [11]. This conversion cycle could be the strategy adopted by the photosynthetic organisms to survive in Space [unpublished results].

Photo II, a multicell fluorescence biosensor. In recent years, chlorophyll *a* (Chl *a*) fluorescence has become so essential in physiological and ecophysiological studies, that all investigations concerning photosynthetic performance of algae and plants are considered complete only if accompanied by fluorescence data. The great success of this technique is attributable to the fact that it gives the possibility of determining the physiological state of photosynthetic organisms, under conditions in which other methods would fail and, above all, in an instantaneous and non-intrusive manner. It means that performing fluorescence analysis, unlike most analytical techniques, does not always require sample preparation steps and therefore direct measurements can be often performed [12].

The basics of chlorophyll fluorescence have been extensively discussed over the last decades. Substantially, when photosynthetic organisms absorb light, a chain of reactions, overall known as photosynthesis, begins. The process starts with the absorption of photons of light by Chl molecules surrounding the two photosystems (PSII and PSI) organized in light harvesting complexes in the photosynthetic apparatus [13]. This creates resonance energy that is transferred to the neighbouring Chl molecules, reaching finally the reaction centre (RC) embedded in the core complex [14]. Consequently, another series of reactions involving different mobile carriers occur, leading to the production of energy rich molecules and reducing equivalents, which are needed to convert carbon dioxide to carbohydrate via the Calvin cycle. In this way, by several physical and chemical mechanisms, radiation energy is transformed into chemical energy. However, not all radiation energy follows this fate, the excess of it being dissipated as heat (around 18%) or re-emitted as fluorescence (1-2% of the total light absorbed)**.** The three processes (photosynthesis, heat production and Chl fluorescence) occur in competition, in the way that any increase in the efficiency of one will produce a decrease in the yield of the other two [12, 13, 15, 16]. Thus, if photochemistry is blocked (for instance, due to the presence of ionizing radiation), the yield of non-photochemical reactions proportionally rises, giving indirect information about the overall photosynthetic performance. Since at room temperature the major contribution to Chl *a* fluorescence comes from PSII, whereas that of PSI is smaller (around 10-25% of the Initial fluorescence F_0), measuring Chl *a* fluorescence also represents a valid tool for indirect investigation on the state of PSII.

In experiment Photo II measures were based on chlorophyll fluorescence induction, also known as fluorescence transient or Kautsky's effect [12, 13, 15].

As shown in figure 3, Photo II is composed of four identical, independent units, each of them powered by two batteries in series (7.5 V). Every unit is composed of two separated modules, each one made up of three optical cells where the fluorescence measurements are carried out.

In each cell, the measurement system is composed of four red light LEDs and an optical fluorescence sensor that provides hourly measurements. The exciting light pulse from the red LEDs is for 6 seconds with an intensity peak at λ=660 nm, inducing the chlorophyll fluorescence; the average intensity of the exciting red lights is ~800 μmol m^{-2} s^{-1}, as measured at the centre of the cells using a quantum radiometer. Before starting each measurement session, the samples are dark adapted for 15 minutes to allow a complete reduction of Photosystem II. A filter is mounted on the top of the optical detector that allows the high-pass transmission of the fluorescence light with wavelengths λ>690 nm. The fluorescence measurements are then digitized and recorded in a non-volatile memory (NVM), where more than 500 measurements can be stored.

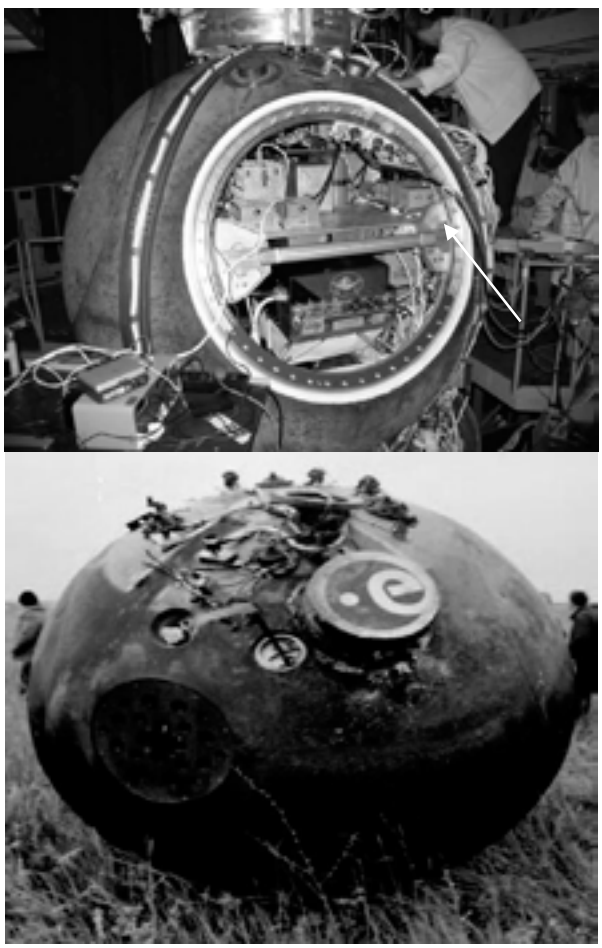

Fig. 2. The above to below: Capsule Foton before flight; with Photo II (while arrow) inside the capsule; Foton after landing.

In each measurement cell the living conditions are provided by two white light LEDs that are switched on continuously for 7 h out of a 24 h period (17

hours of dark) and guarantee the photoperiod necessary for the survival of the samples.

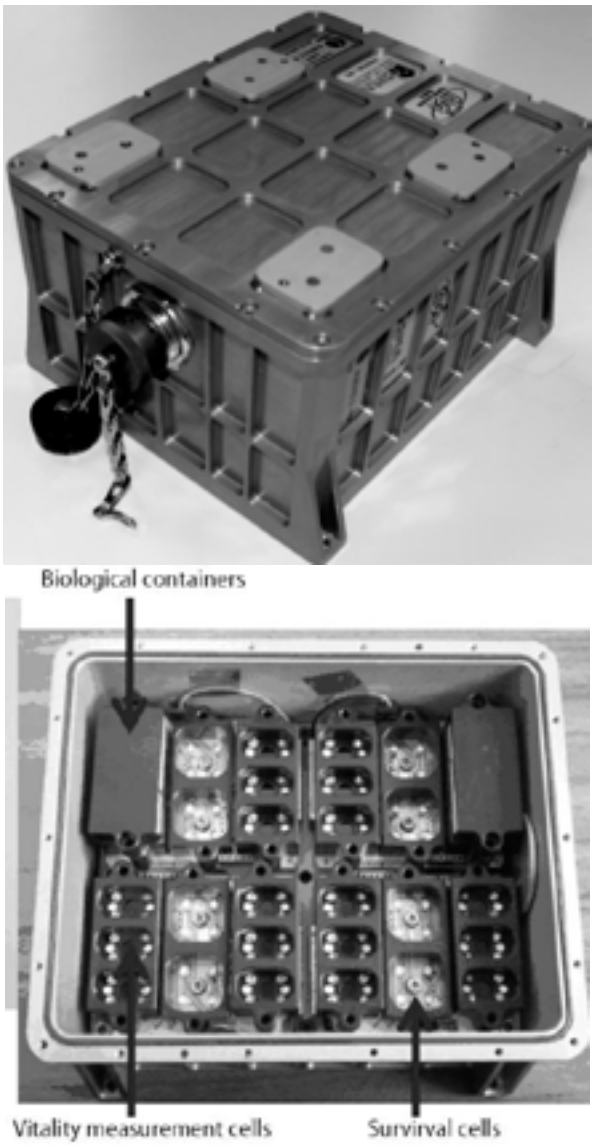

Fig. 3. The Photo II fluorimeter used in Foton Space missions; the main components are shown and highlighted. External box was built by Kayser-Italia, while electronics were built by Italian companies: Biosensor, Carso.

The intensity of the white lights can be set up to a maximum of 250 $\mu molm^{-2}s^{-1}$ as measured at centre of each cell.

The material used for the containers of the biological samples is Delrin and the transparent window exposed to the light is made of Polycarbonate and a steel frame is used to seal the cell. Black Delrin is compatible with the biological material and guarantees optical isolation among the cells. A gasket of silicone provides perfect sealing, thus avoiding any contamination of the biological material after being placed in the container.

Finally a set of eight independent thermometers to measure the temperature inside the device complete the electronics of Photo II.

2.1 Photo II Electrical and electronic system

Space is a very hostile environment for electronic components. The presence of radiation of different types, intensity and energy can cause various effects some of which are potentially dangerous for the mission [17, 18].

The radiation effects due to long-term exposure in the space are known as total dose effects. This accumulates energy (in the form of ionized atoms) over time in the target material leading to an increase of leaking currents in isolators and transistors, charge trapping in MOS gate oxide (potentially leading to transistor failure) and loss of internal chip isolation.

Another effect of radiation is caused when a single high-energy particle hits an electronic device. It leaves behind a column of ionized material that is like a conducting wire suddenly inserted into the device disturbing the currents and electrical fields inside it. Therefore, electronic systems for Space applications must be carefully designed. Shielding is crucial but it is not enough to resolve just this issue; devices, known as radiation hardened or called “Rad-Hard”, must be used to withstand conditions in Space [18].

NASA and ESA have programs for testing and cataloguing components for Space missions. These are the NASA Electronics Parts and Packaging Program (http://nepp.nasa.gov/index.cfm) and the European Space Components Coordination (https://spacecomponents.org/). Lists of approved components are published periodically (GFSC Preferred Part list in NASA and ESC Qualified Parts List in ESA) and these were used as the basis for the development of Photo II.

The electronic system is composed of three specific function cards. The Photo II control board is based on a 32-bits x486 AMD processor (Elan SC410). This processor is a low power CPU and works at a

frequency of 33MHz. Externally a 2MB flash program memory and a 8MB RAM memory have been provided.

3. NIGHT VISION

One of the main problems for astronauts exposed to long-duration space flight is the exposure to ionizing radiation and the consequent oxidative stress. One of the organs affected by ionizing radiation is the human retina. Moreover, the continuous changes in light due to the movement of the International Space Station (ISS) can lead astronauts to experience various dawns and sunsets over 24 hours. These phenomena cause troubles and difficulties in maintaining the rhythm of sleeping and the vision is particularly difficult in missions external to the ISS.

Lutein (substance found in vegetables that protect against cell damage) and zeaxanthin (substance usually found in yellow/orange fruits and vegetables, as well as egg yolks) are the pigments present in both the macula and lens of the human eye which are also referred to as macular pigments (MPs). They belong to the family of xanthophylls (yellow and orange pigments found in plants, animal fats and egg yolks) which are oxidized derivatives of carotenes, including several compounds. Both carotenes and xanthophylls belong to a class of polyisoprenoids (synthetic molecules). MPs' effects on the human body include the improvement of visual function, and the protection from photo-induced damage, as they act as filters for blue light and shield short-wave radiation.

Epidemiological studies have shown a strong correlation between the levels of lutein and zeaxanthin in eye tissues, serum and blood plasma, with a reduced incidence of oxidative stress associated with age and macular degeneration pigment.

MPs cannot be synthesized by the organism and must be introduced via the diet. There are other xanthophylls that also play an important role in protecting visual apparatus. The unicellular alga *Chlamydomonas reinhardtii* possess only one chloroplast that is in contact with an orange organelle called eyespot; similar to the human retina. As the human retina, the algal eyespot presents macular pigments involved in perception of light and a similar organization. Other algae with similar eyespots include *Chlamydomonas nivalis* and *Haematococcus pluvialis*.

This project proposes the study of resistance to ionizing radiation of algae and *Chlamydomonas reinhardtii* genetic mutants that accumulate various quantities of macular pigments in the eyespots. The extracts of eyespots will also be immobilized in alginate (salt from alginic acid) and their antioxidant effects will be evaluated for future nutrition programs in space. These immobilized matrices will be analyzed by means of X-ray (powder X-ray diffraction, XRD) to study the relationship between organization and functionality of the eyespots. One of the organs mostly affected by cosmic radiation is the visual apparatus; in particular, the central and peripheral photoreceptors of the retina. The global vision in astronauts is disturbed in the perception of colors and movements. The result is that the vision during the night exploration is particularly disturbed. Recent studies on the ISS suggest that a unique ionizing heavy particle can hit one or plus photoreceptors in the retina, including damage to other eye tissue, such as the lens. The mechanism of oxidation at retina level is not known in detail. One hypothesis is that the damage is generated from a genetic damage in the lens epithelial cells, including the destruction of normal cellular life cycle, apoptosis (cell death), abnormal differentiation of cells and cellular disorganization.

The Department of Aviation Medicine is particularly interested in increasing the visual efficiency of astronauts as the number of working hours is reduced as a result of reduced visual fatigue. To understand what happens inside astronauts eyes, scientific literature proposes several models *in vivo* and *in vitro* as to study the high ocular pressure in the retina and in the optical nerve, which occurs during oxidative stress. For the in vivo studies, an empiric model based on guinea pigs is used, causing ocular hypertension by injecting methylcellulose in front of the eyes. In guinea pigs, or humans affected by oxidative stress, eye neural tissue degenerates with a cell death program.

Previous investigations were conducted which studied the effects of ionizing radiation on the photosynthesis of several microorganisms. Various radioactive sources and facilities were used in radiation simulation studies. The results provided useful information on the radiation environment in Low Earth Orbit (LEO) monitored during the Foton M3 (robotic spacecraft used by Russia and the

European Space Agency (ESA) for research conducted in the microgravity environment of Earth orbit) mission in 2007. The data was obtained by a couple of active spectrum-dosimeters that measured in real-time, the deposited energy spectrum by the incident ionizing particle, the particle flux and the absorbed dose behind different shielding. As a whole, this analysis indicated very quiet and low solar activity being the total dose measured on the silicon detectors. Green alga was found to be the less sensitive photosynthetic microorganism tested. Previous experiments demonstrated that the consequence of space stress on microorganisms seemed to be inversely correlated to cell dimensions. It appeared that large cell cross-sections, with their content of lipids, antioxidants and enzymes; could partially shield internal structures; however, other possible factors could account for the higher sensitivity observed in green algae.

In order to unravel the response of the photosynthetic apparatus to real space conditions, stratospheric balloon flights and Foton M2 and M3 space missions were successfully exploited. The organisms observed to be tolerant to ionizing radiation in the simulation studies, were used for the real space condition experiments. Investigators found that the effect of the ionizing radiation on the activity of PSII (Photosystem II - a protein complex) was enhanced in space, compared to that observed in ground based facilities. The PSII activity, the growth rate and the survival ability of the tested organisms were altered even at low doses.

3.1 Night vision, biomediator's survival system

Eyespots and Macular Pigments Extracted from Algal Organisms Immobilized in Organic Matrix with the Purpose to Protect Astronaut's Retina (Night Vision) is a study on the response of microalgae strains (that contain eye spots similar to the human retina) to space radiation in order to obtain results applicable to future nutrition programs for astronauts.

The instrument has been develop for contain the samples of different mutants in total are 36 samples divided in three containers with three levels (see figure 4), each sample is illuminated with a white light source at 13 $\mu mol*m^{-2}.s^{-1}$ and one photoperiod of 7 hours of light and 17 hours of dark; the instrument have an autonomy of 20 days. The instrument has been built in accord at the standards NASA. The electronics have been developed in function at the passive component for avoid problem of cosmic ray.

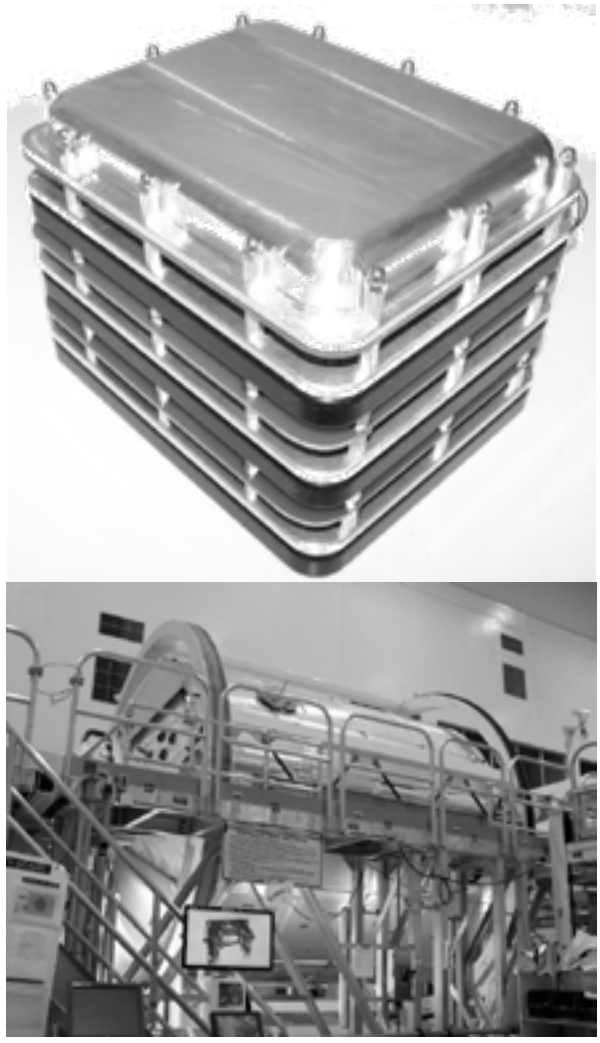

Fig. 4. The Night vision instrument (above) used in the module MPLP (below). External box and electronics was built by Biosensor this is an Italian company.

Furthermore, in phase of analysis of the instrumentation, Night Vision was tested by off gassing at ESA Labs

4. BIOLOGICAL ORGANISMS IN SPACE

Photo II monitored automatically the photosynthetic activity of 24 samples for more than 20 days,

measuring hourly the fluorescence curve for each sample and determining the main parameters of the curve: F0 and FM, minimal and maximal fluorescence intensity, respectively; FV/FM where FV=FM-F0 is the variable fluorescence and the area below the curve [12, 13].

Twelve different strains of the unicellular green algae *C. reinhardtii* were placed in the 24 measurement cells. Some of the samples employed in the experiment on board Foton are site-directed mutants of the D1 protein, and each mutant is characterized by a single or double substitution in the aminoacidic chain of the protein [19, 20, 21, 22].

Some of the strains used in Photo II experiments were previously selected after irradiation tests conducted in ground-based laboratories. The mutants of *C. reinhardtii* were exposed to γ-rays, energetic neutrons, protons and heavy ions, and the strains selected for Photo II were those which proved to be more radio resistant to this treatment [22].

The figure 5 shows a set of fluorescence induction curves measured in Space by the Photo II device for three different photosynthetic samples. Fluorescence is measured for 6s, that is the duration of the excitation pulse provided by red light LEDs. Some of the parameters which identify each curve are indicated in the plot (see figure 5) the maximum fluorescence F_M, which is measured directly and the initial fluorescence F_0, which is determined by extrapolating the curves to t=0. The F_V/F_M parameter, derived from F_M and F_0, provides a measure of the photosynthetic efficiency of the organism.

When the F_V/F_M parameter is constantly monitored over a long period of time as it was during the Foton flights, gives information on how and how much the photosynthetic performance is responding to Space stress [9, 22]. The study showed that the effect of ionizing radiation on the PSII function depends on the intensity of the photosynthetically active radiation (PAR) present [9]. In the dark and under relatively intense PAR, the damage to PSII induced by the ionizing radiation increased, while this effect was negligible under weak light. This synergistic effect could be due to a phenomenon similar to photo inhibition; both ionizing radiation and high light intensities are known to cause the formation of free radicals which are harmful for the photosynthetic apparatus. One of the reasons that caused the photo inhibition of PSII and the chain of reactions involved in the D1 turnover is thought to be the accumulation of singlet oxygen at the PSII site. The D1 protein could play a potential role in strengthening the resistance of PSII to ionizing radiation [9-22].

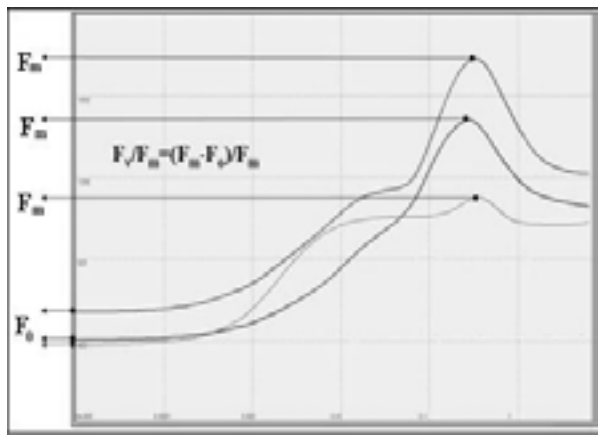

Fig. 5. Fluorescence curves measured by Photo II fluorimeter for three different mutants of the photosynthetic organism *C. reinhardtii*.

The synergetic effect of radiation and light on the photosynthetic apparatus should be seriously taken into consideration in relation to the question of the survival of photosynthetic organisms in Space since bio regenerative life-support systems can be severely affected by this environment. This emphasizes how important it is to continue studies which can demonstrate the actual effect of complex Space radiation on the photosynthetic process and apparatus.

5. FROM SPACE TO EARTH: A DUAL USE TECHNOLOGY FOR FLUORESCENCE BIOSENSORS

The adaptation of a Space oriented instrument for application on Earth offers huge possibilities of optimization and expansion.

As rad-hard components are not necessary, the design of the electronic system is more flexible. In the case under study, the use of commercial, highly integrated last generation microcontrollers eliminates the need of a microprocessor without causing a loss of computing power and greatly reduces the number of components and the cost of production. Other important functions can be

implemented such as a friendly user interface, data communication systems.

Less weight and more power availability are involved in applications on Earth than in Space. Heavy shielding and protection material is no longer necessary. The batteries can be completely eliminated or in the case of portable applications replaced with commercial devices. This means that an automatic fluidic system can be utilized for sample handling, and offers the possibility of using more measurement cells at different excitation and emission wavelengths, thereby opening the way to new applications involving the use of various biological materials.

The technological transfer of Photo II allowed an optical biosensor platform for multi-analyte monitoring to be developed. Two examples of technology transfer of the spatial fluorimeter are discussed: OPTICBIOMulticell, a biosensor platform for environmental monitoring and SensDNA, a patent for the analysis of labelled DNA, RNA, oligonucleotides and proteins. Moreover, related patents on the field of biosensors are discussed.

The importance of studying photosynthetic organisms consists on the concrete possibility of using them as probes for environmental monitoring. This applicative possibility is substantially due to their sensitivity toward different toxic compounds, such as herbicides or heavy metals [2-4]. The presence of these compounds affects photosynthetic electron transport in a concentration-dependent manner, causing different effects according to their specific site of action, but always with the same consequence: a change in the yield of Chl *a* fluorescence. From this point of view, therefore, this technique is able to reveal any damage produced on the photosynthetic apparatus, independently from the specific target site of the toxicant producing that damage. An additional advantage of this technique is that the results can be achieved in a faster way than other procedures, such as algal growth tests [23-25]. Moreover, chl *a* fluorescence can be an easy tool for the pre-screening of toxic compounds, useful for properly addressing further GC and/or HPLC analyses [7, 8].

The OPTICBIOMulticell (Biosensor S.r.l., Rome, Italy, www.biosensor.it) is a multibiomediator fluorescence instrument equipped with 96 LEDs with different emission wavelengths for fluorescence excitation and 24 silicon photodiodes and optical filters for fluorescence emission measurement. The invention provides biosensors based on microalgae for the determination of the presence of toxic compounds in a sample, characterized by the high sensitivity and specificity of genetically modified algae thereof with respect to the target toxic substance [7, 23, 24]. Each photodiode provides a spectral response in the range of 300-1100 nm, and the fluorescence peak can be selected by different band-pass optical filters. This instrument was used to automatically calculate fluorescence parameters. Variation in the photosynthetic activity of the samples, in response to environmental or chemical changes, such as the presence of photosynthetic herbicides or pollutants was determined by fluorescence measurements [6, 7, 8, 23, 24].

A variant was described by McCarter and Jenkins in the patent “Water monitoring systems” that applied a complex fluidic device for monitoring water by algae cultures based on fluorescence” [26]. Another variant is described by Green L and Pritest in “Portable biosensor apparatus with controlled flow” with a developed system composed of a fluidic cube comprised of a vent cap, an isolator and a waveguide with a fluid manifold. The biosensor is designed to simultaneously process multiple samples for a variety of analytes [27].

The recent patent from Moraleda *et al.* entitled “Biosensors based on microalgae for the detection of environmental pollutants” does not use the property of fluorescence emission by algae but the detection is through a luminescent compound, the emission of which depends on the amount of oxygen produced by algae in the medium [28]. A similar approach is also reported by Varsamis *et al.* [29] in which photosynthetic membranes isolated from higher plants and photosynthetic microorganisms, immobilized and stabilized, can serve as a biorecognition element for the biosensor. The inhibition of photosystem II causes a reduced photoinduced production of hydrogen peroxide, which can be measured by a chemiluminescence reaction with luminol and the enzyme horseradish peroxidise, and used for pollutant detection.

Other biosensor applications of the Photo II device were envisaged, such as in SensDNA [5]. This device is a portable, modular, automatic and computer-operated device allowing fast and sensitive measurement of the fluorescence emitted by various fluorophores, capable of binding to

biological macromolecules in solution or being immobilized on specific media. The instrument can work on a wide range of wavelengths and make simultaneous and/or groups of independent sample measurements treated with different commercially available fluorophores. The sensor consists of four independent modules, two static and two dynamic, each of which contains six cells, for analyzing up to twenty-four samples, the system is easily extensible to eight modules for a total of forty-eight samples; each module consists of two units mounted on top of the instrument. The upper unit is independent and interchangeable, and contains cells to house the samples.

The lower fixed unit is the optical sensing element; containing the source of light emission (LEDs) and the optical system for capturing emitted fluorescence. The tool also has a temperature control system ranging between 25 and 50 °C, useful in the fluorescence intensification of some fluorophores. SensDNA can be applied to the medical, biological, toxicological and pharmacological field [5].

6. CURRENT AND FUTURE DEVELOPMENTS

Photo II was developed to determine the resistance of *C. reinhardtii* mutants to Space conditions. Different algae strains were tested to assess their potential application as a source of food and oxygen during long term Space exploration missions. Photo II successfully completed its mission thanks, in particular, to the Space-oriented design of the electronic system.

The less harsh conditions on the Earth allowed the architectural structure to be modified, thereby improving the functions of the instrument and lowering its cost. Also the fluorescence methods used by Photo II demonstrated its potentialities in biosensor applications - particularly, in the case of pollutant detection in water – stimulating future developments in this field. Biosensors have emerged as a promising technology especially for applications where continuous monitoring is required. In the last few decades, progress in biochemistry, molecular biology, immunochemistry and the development of microelectronics and nanotechnology, has allowed biosensor technology to move out of the laboratory towards commercial applications. SensDNA and OPTICBIOMulticell (see www.biosensor.it) are two examples of Space-Earth technological transfer of this instrument.

Future research will have to focus on improving the specificity of the signal to specific pollutant subclasses. Steps towards different algae combinations, sensitive to different analytes, should be taken in another effort to improve photosynthetic biosensors. For this reason, research should be carried out to find new engineering algae, robust and with long half-lives. By genetic engineering we already developed mutants extremely resistant to Space conditions that can be useful for biosensor applications [22 and unpublished data].

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