Portable instruments based in multiband fluorescence transducers for biosensing applications

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Abstract

Two prototypes of the miniaturized biosensor-based optical instrument has been designed and fabricated for fluorescence measurements of several biomediators in series or only one, with applications in environmental monitoring and agrofood analysis. It is a multicell system, every cell is made up by two modular sections: the bottom compartment with optical LED light excitations and a photodiode detector for fluorescence emission capture, and the top biocompatible compartment where the biosample is deposited.

The prototypes has been implemented with geneticalIy modified algae that were employed in the instrument experimental testing by monitoring pesticide pollution in water. Pesticides modify the photosystem II (PSII) activity in terms of fluorescence quenching. Results from measurements employing several C251 mutants and three different pesticides at increasing concentrations and incubation times are presented and discussed.

Key Words

Sensor, biosensor, biomediator, fluorescence, fluorimeter

Resumen

Dos prototipos de micro instrumentos ópticos basados en biosensores han sido diseñados y fabricados para mediciones de fluorescencia de varios tipos de biomediadores en serie o individuales, con aplicaciones en monitoreo del medio ambiente y análisis agroalimentario. Se trata de un sistema multiceldas, cada celda está compuesta por dos secciones modulares: en la parte inferior, un compartimiento con excitación óptica de luz LED y un fotodiodo detector de emisiones de fluorescencia, y en la parte superior, un compartimiento biocompatible donde se deposita la muestra biológica.

Los prototipos se han aplicado con algas genéticamente modificadas, que fueron empleadas en instrumentos para ensayos experimentales en monitoreo de pesticidas en la contaminación del agua. Los pesticidas modifican la actividad en el foto sistema II (PSII), en términos de disminución de la intensidad de fluorescencia. Se presentan los resultados de las mediciones que emplean varios mutantes como el C251 y tres diferentes pesticidas en el aumento de las concentraciones y tiempos de incubación.

Palabras Claves

sensor, biosensor, biomediador, fluorescencia, fluorimetro

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Introduction

Fluorescence emission excited by a proper light wavelength is a widely studied phenomenon and a commonly used technique for plants physiology characterization and monitoring of photosynthesis activity. Several optical devices and measurement systems have been employed for this purpose: a Fraunhofer line discriminator together with a low-pressure cell containing oxygen, coupled to a photomultiplier tube [1]; a filtered CCD camera detector that captures a complete image of the sample (e.g. FluorImager, Molecular Dynamics Inc.); laser-induced fluorescence spectroscopy coupled to CCD imaging especially in remote sensing applications [2,3].

Some of the authors of this work previously reported about a biosensor bench apparatus for herbicides detection in which an algal mutant was deposited in a cell and chlorophyll fluorescence excitation and emission detection was performed by a modified plant efficiency analyzer from Hansatech Ltd. [4, 5, 6].

In the same work an alternative parameter in fluorescence signals deconvolution was adopted for quenching analysis, due to the strong variations exhibited: the area between the F0 line (defined as minimal fluorescence yield in the dark-adapted state) and the intensity-time curve response. This same analysis technique is employed here in the case of measurements with photosynthetic microorganisms. In fact several types of biomediators can be measured by the transducers described in this work, which are multipurpose portable instruments featuring a low size and weight, modularity and flexible optical heads, suitable to a variety of applications, from agro-food to biomedicine and environmental analysis. Plants, photosynthetic bacteria and cell samples can be monitored thanks to the integration of different excitation light sources and fluorescence emission detectors[7]. Two systems architectures have been developed and implemented into two multiband instruments.

Fluorimeter for Space Applications

The first one is multicell thus allowing the measure of 10 biological samples at a time, with two modules of 5 vessels each mounted onto independent optical modules, made up by LED sources in the red and blue spectral range, a photodiode detector and an interferential band pass filter, tuned on the desired fluorescence intensity peak. In case of photosynthetic microorganisms, white survival LEDs are provided. Fig.1 shows a picture of the instrument lodged in a special container complying with space compatibility and safety issues: this flight model of the multiple transducer was employed to test microgravity stress induced variations of the physiological activities of Chlamydomonas rehinardtti algae during the European Space flight mission. Its weight is 0.200 gr and dimensions are 100x100x150 mm.

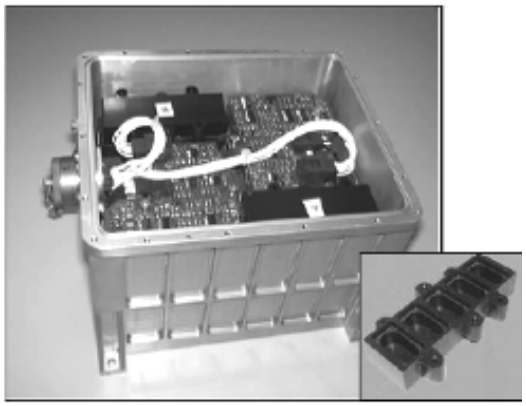

Figure 1: Picture of the multicell fluorimeter employed in the space flight (the cells module is shown in the inset).

Multiband Fluorimeter for Biosensoristic Applications

The second transducer allows for even more versatility with a compact optical head containing 12 optical fibers (1mm diameter, aperture angle of 55º) all connected to the biological monocell, bringing the excitation signals in from 6 LEDs and collecting the fluorescence emission towards 6 photodiode detectors. The LED sources provide the following wavelengths in nm: 400, 450, 500, 550 for DNA (use the techniques ; 600, 650, 700 for plants and 880 for bacteria). Emission wavelengths range from 500nm to 800nm. Fig.2 shows a photograph of the optical head with the cell inserted on the top and the fibers coming out with 20º and 30º inclination.

The instrument size is 70mmx70mm and is equipped with internal lithium batteries. The

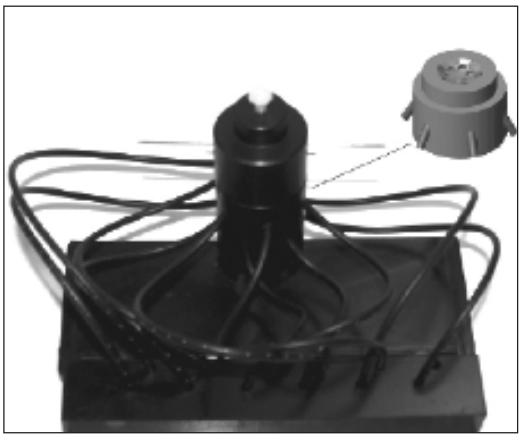

Figure 2: Picture of the multiband fluorescence transducer with a design detail of the optical head with excitation and emission optical fibers terminations.

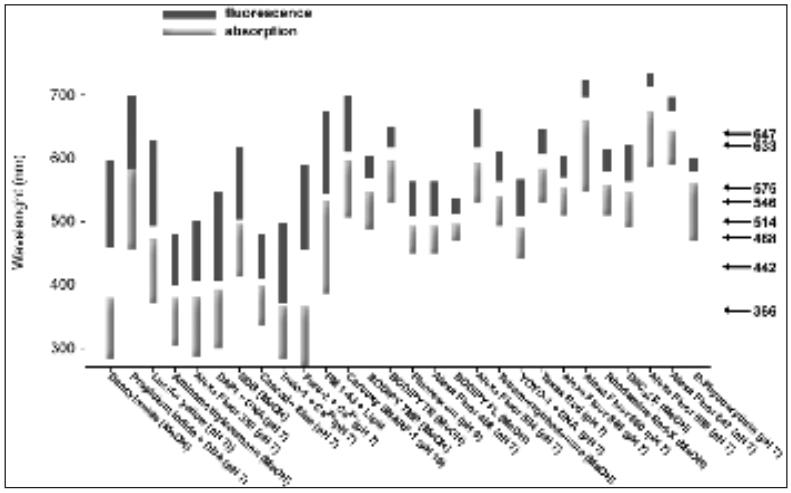

Figure 3. Indication of absorption and emission wavelengths of common fluorescence markers, for the DNA Fluorescence Measurement for the

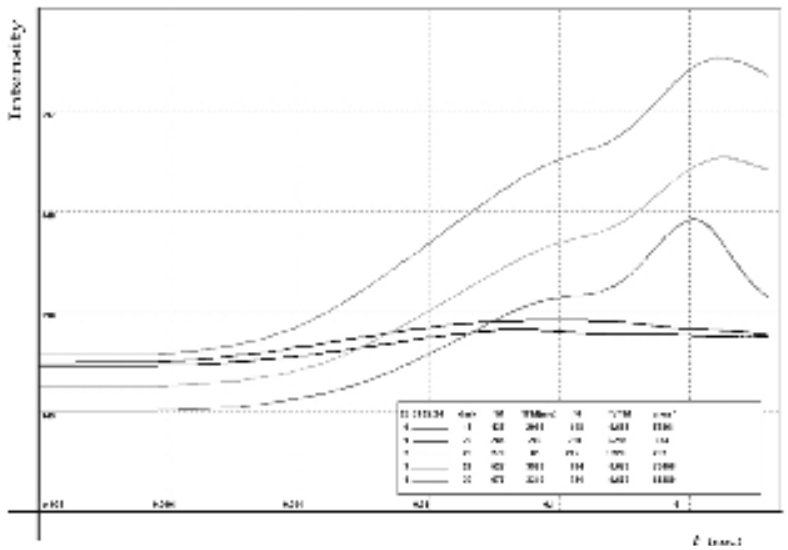

Figure 4. Screen shot of a real time fluorescence intensity measurement of 3 different biosamples. Quenching is shown by the 2 darker lines on exposure to diuron.

goal of the device, is the fluorescence measurement with fluorophore marked method; in the Fig. 3 summarizes the most commonly used DNA fluorescent markers and highlights the advantage of the multiband transducers.

Experimental Testing

Experimental biosensing tests have been preliminarily carried out by adding an increasing concentration of pesticides to cells containing different C. rehinardtti mutants. In Fig.4 the inhibition induced by diuron is clearly shown by the two quenched curves: data reported in the legend refer to the parameters Dark, TFM (ms), F0, FV/FM and the Area.

Where the typical parameters, utilizes in fluorescence are:

Dark = measurement of fluorescence, when isn't present the excitation light

TFM : time of fluorescence measurement

F0: measurement of base fluorescence or bias

FV : measurement of variation fluorescence

FM : measurement of maximum fluorescence

FV/FM : measurement of relation fluorescence (normalization)

Area: Analysis in the area under the curve

The variables are describe in the Fig.5; that represent the fluorescence response to 3 pesticides at increasing concentrations.

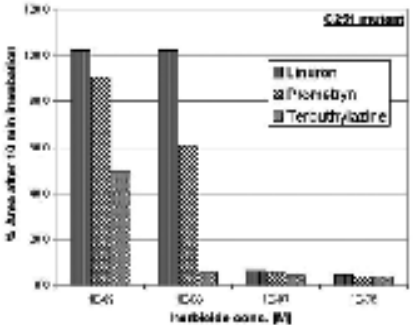

Figure 5. Experimental results of fluorescence response of the C251 mutant of Chlamidomonas algae to 3 different pesticides at increasing molar concentrations.

Conclusions

An innovative miniaturized fluorescence detector system has been designed and fabricated with special attention towards modularity, flexibility and low cost. The fluorescence of ten different biomediators in series can be contemporarily excited and measured under the influence of different chemical or environmental compounds, due to LED light sources and photodiodes coupled to interferential band pass filters, directly integrated into the optical compartment of the measurement cell. Therefore the instrument allows one to perform simultaneous and physiological multiparametric analyses. Fluorescence can be an intrinsic property of the biological material or otherwise induced by specific fluorescent probes (e.g. for DNA and proteins). Therefore, the new sensor has a large applicability in biosensor technology. The instrument performance has been validated through several measurement campaigns in which six different photosynthetic biosamples were deposited in the cells (C251 mutants of the photosynthetic alga Chlamydomonas reinhardtti) and up to three pesticides were administrated (terbutylazine, prometryn and linuron). Measurements were performed at different concentrations and inhibitor incubation times.

The minimum fluorescence signal detected by the instrument was the one emitted by 1 μg of biomediator and the minimum detected inhibitor concentration was 10−9 or 10-10M, for certain classes of pesticides (view Fig 5).

Experimental results proved to be comparable to previous ones obtained with a commercial apparatus, thus providing the miniaturized system with sufficient reliability to be employed in agrofood analysis and environmental monitoring.

Acknowledgments

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