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Application of an Ultra-Weak Bioluminescence Measurement System for *Escherichia coli* Detection in Sanitary Control

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***Abstract*—Detection and measurement of ultra-weak photon emission (UWPE) from biological samples is a promising tool with potential use in several fields such as agriculture, environmental science, food science and biomedicine. A measurement system especially designed to detect UWPE, and an application in sanitary control of natural water resources is presented here. The system was implemented based on a dark-chamber with a photomultiplier module (PMT) cooled by a microprocessor controlled thermoelectric device coupled. The PMT detects the UWPE from the biological sample under measurement. The performance evaluation of the measuring system in terms of dark-noise and bacteria detection was performed in order to assure that it is able to realize UWPE measurements for the proposed application. The samples under test were comprised of a series of 3 control cultures of standard *Escherichia coli* strain, used as control, and other 3 water samples collected from a river close to a metropolitan area in Brazil. The comparison between the control and test samples has shown that the proposed application is feasible for *Escherichia coli* detection tests in water samples from natural water resources to assure the evaluation of their microbiologic quality.**

***Index Terms*—Ultra-Weak Bioluminescence, Escherichia coli, Sanitary Control.**

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I. Introduction

ULTRA-WEAK photon emission (UWPE) detection and measurements has been a subject of research by many groups, and it has a potential use in diverse areas such as agriculture, environmental sciences, food sciences, and biomedicine [1].

UWPE is present in all biological processes, and it only ceases after organism death. Its spectrum ranges from the ultraviolet to the near-infrared, from around 350 to around 850 nm, and its intensities typically are from tens to thousands photons per $cm^2 \cdot s$ [2], or from 10^{-20} to 10^{-15} $W \cdot cm^{-2}$ [1].

The UWPE can be divided in spontaneous and stimulated emission or delayed luminescence (DL). DL is produced after stimulating a biological sample by some type of physical or chemical stress, or by a light source stimulus, and its response is quite distinct for biological samples from non-living samples, as demonstrated by Zeiger in [3]. While the spontaneous emission presents intensities from tens to hundreds photons$\cdot cm^{-2} \cdot s^{-1}$, or from 10^{-20} to 10^{-17} $W \cdot cm^{-2}$, the stimulated emission ranges from hundreds to thousands photons per $cm^2 \cdot s$, or from 10^{-17} to 10^{-15} $W \cdot cm^{-2}$.

The UWPE phenomenon was first observed by Alexander Gavrilovitch Gurwitsch in the 1920's [2], when he theorized the existence of a kind of radiation, which he called mitogenetic radiation. Only in 1951, Strehler and Arnold, using the recently invented photomultiplier tube (PMT), and using as biological samples a kind of algae [4] positively confirmed the existence of the mitogenetic radiation. Short after, in 1954, Colli and Facchini made the first UWPE measurements from seedlings using a PMT [5, 6].

Since then, many groups studied the UWPE correlation between the development capability and germination rate of different seeds: barley [7], rice [8], soya [9]. In addition, a general patent was registered in 2001 [10].

In the case of microorganisms, the UWPE from *Escherichia coli* [11, 12, 13, 14, 15], *Lactococcus lactis lactis* [14, 15],

Serratia mercescens, *Enterococcus faecalis*, *Lactobacillus plantarum*, *Proteus vulgaris*, *Deinococcus radiodurans*, *Vibrio fisheri* [15], were also studied. Other applications are studied the defense mechanisms of microorganisms under physical or chemical stress, and the source of light emission [16].

The most accepted theory about biophotons origin points to cellular structures and its metabolism from enzymatic and non-enzymatic reactions, where oxidative stress with formation of reactive oxygen species take place [17].

In order to detect and measure UWPE/DL a specialized instrumentation is required. The central component is a PMT module that must provide photon counting operation with low dark-count noise [18].

UWPE experiments demand specific photon-counting systems, sometimes suitable for each particular application. For instance, Tudisco *et al*. proposed a method to perform optical biopsy of human cells using DL with very short time delay from the light excitation to the starting photon counting. They showed that such measurement is possible with the use of a specific electronic control that inhibits the PMT, and let the start photon-count just 10 μs after finishing excitation [19].

For DL measurements of single dry soya seed, using time resolved spectral analysis, the characterization needs some schema for insertion and removal of optical filters [20]. To do it a laser beam is sent by a bifurcated optical fiber to the dark chamber, where a single seed is placed. When the DL signal returns, it passes by optical filters before being detected by the PMT, which is kept cooled at -20°C. A setup with controlled water injection into the dark chamber, to measure chemiluminescence (CL) of rice seeds during early imbibition is presented in [8]. In this setup, the formation of singlet oxygen ($^1O_2$) could be correlated to the vigor of the rice seeds. Seeds that were stored shorter time produced stronger CL.

The UWPE measurement system here described is based on a previous work [18]. It is intended to be of relatively low-cost, offering resources as automatic control of the PMT power supply, illumination capabilities for DL measurements, sample and PMT temperature control and optical filters insertion capability. Once the system was constructed, it was possible to evaluate it in terms of dark-count noise performance. Results showed that the proposed instrumentation presents the adequate levels of dark-noise, and resulted in high SNR, when used to measure UWPE from bacteria cultures, suitable for application in sanitary control.

II. Materials and Methods

A. The photon-counting setup

The block diagram of the measurement system can be seen in Figure 1. The main element of the system is the PMT module: H6240-01, from Hamamatsu Inc., spectral response from 185 to 850 nm, incorporating all circuits needed to provide photon-counting capability, such as low-level dark-noise PMT, high voltage circuit, amplifier, pulse discriminator, and pulse conditioning, delivering TTL pulses as the output response to photons incident on photocathode.

The PMT provides counting pulses that are received by a National Instruments acquisition board (NI-USB6008), connected by USB interface to a personal computer. A virtual instrument, designed in LabVIEW®, controls the acquisition process, the PMT module power supply, and the illumination capabilities to be used in the DL experiments.

The instrumentation provides all needed features when dealing with UWPE, such as:

a. Control of acquisition parameters and devices using a customized LabVIEW® virtual instrument;
b. Light excitation capabilities to be used in DL experiments; for implementing it a ring illuminator is fixed above the Petri's dish inside the dark chamber, with a light source coupled to it through a fiber optic cable;
c. Spectral analysis capability by using a set of seven optical filters, which can be selected from a rotatory wheel, allowing insertion of the selected filter in the path between the sample and the PMT window;
d. Possibility of warming the sample by using a heating resistance element outside the dark-chamber and just below the surface where the Petri's dish rests;
e. Scheme to maintain the PMT module cooled at an appropriate temperature in order to stabilize and reduce dark-noise, using a Peltier plate having a water cooler scheme to better efficiency.
f. In order to prevent a possible damage to the PMT, when it is cooled below room temperature, and a condensation may occur, a quartz block was added in the access window located between the dark chamber were the samples are placed and the PMT.

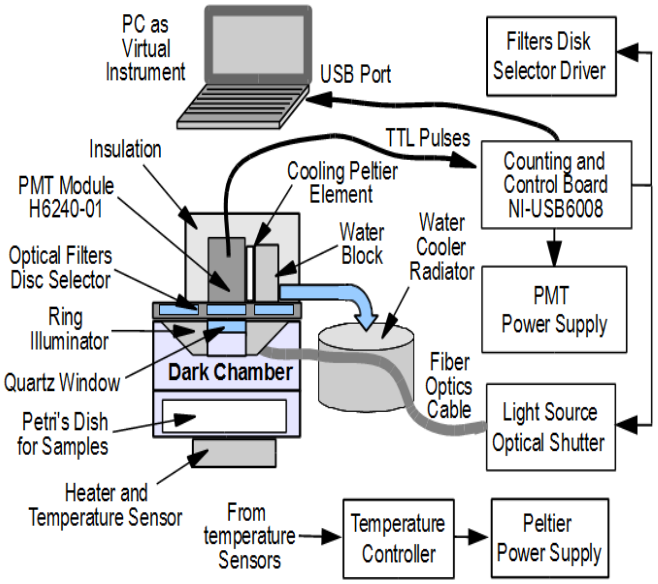

Figure 1. Block diagram of the ultra-weak photon emission data acquisition system showing its constituents elements.

B. The Virtual Instrument

The front panel of the virtual instrument is shown in Figure 2, where one can see the UWPE emitted by *Escherichia coli* sample growing for 24 hours inside the chamber.

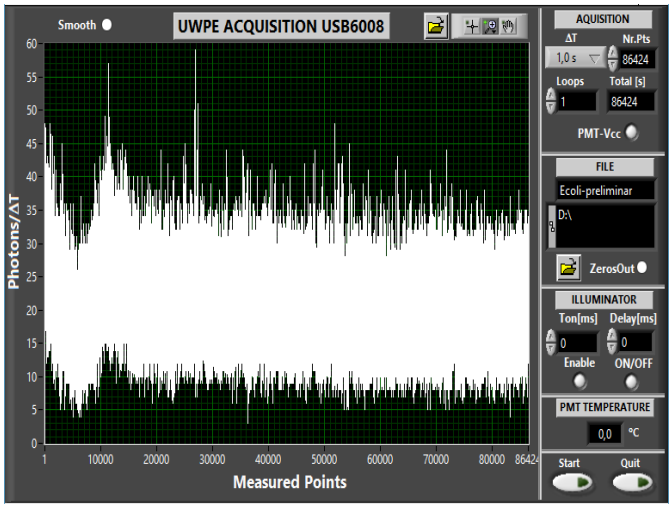

Figure 2. Front panel of the virtual instrument of the system showing an experiment with *Escherichia coli* culture inside the dark chamber for 24 hours.

At the right side of Figure 2, it is shown the setting controls for the acquisition process: photon counts integration time, number of measuring points, and loops for automatic data file saving.

At the bottom, right side of Figure 2, it is shown the PMT temperature monitor, and the light excitation control: duration in seconds and delay for starting the acquisition process after stimulation.

C. Dark-Noise Performance Evaluation

In order to evaluate the dark-noise performance of the dark chamber, some dark-count noise tests were performed during 1 hour each, at 3 different PMT temperatures: 5°C, 10°C and 15°C. The dark-noise measurement is related to the background photon emission from sources as thermal, cosmic rays, and terrestrial emissions.

For the analysis of those dark-noise count measurements, an integration counting time of 1s was chosen, and the final data count smoothed by averaging theirs 100 adjacent samples.

D. Measurement of Spontaneous Ultra-Weak Luminescence of Standard Escherichia coli Cultures

Escherichia coli culture samples were prepared by using a standard strain of that bacterium inoculated in EC type nutrient medium inside an 8 cm diameter Petri's dish.

Three photon-counts experiments using the *Escherichia coli* samples were conducted, always at 37°C and during 24h inside the dark chamber, using an integration counting time of 10s for the counts acquisition, differently of dark noise count measurements in order to generate a smoothed temporal data, and those acquired data counts were smoothed again by offline averaging theirs 100 adjacent samples.

E. Measurement of Ultra-Weak Luminescence of River's Water Cultures

River's water samples were prepared using500 ml of water collected from the Palmital River in Colombo/PR, Brazil. When handling the river's water samples any possible external contamination was avoided. Afterwards, some 1 ml samples of that water were arranged inside test tubes and stored at 10°C.

The multiple tube technique was applied with the purpose of knowing the most probable number (MPN) of bacteria in the river's water sample, and the NMP of110 microorganisms per 100 ml was found [21]. Subsequently, aliquots of 1 ml of river's water sample were added to 10 ml of EC medium, and these inoculums were inserted into an incubation chamber, where it stayed for 24 hours (overnight) at 37°C.

The incubation allowed the microorganisms grow up to the beginning of the stationary stage. After the overnight period, the river's water samples were put into an 8 cm Petri's dish, and warmed up at 37°C, and then left inside the dark chamber for the UWPE measurement.

Following the procedures described above and using the same parameters from the *Escherichia coli* sample, three photons-counts experiments using the river's water samples were conducted, always at 37°C and during 24h inside the dark chamber.

III. Results and Discussion

A. Performance Evaluation - Dark Noise

As mentioned before the measurement system was evaluated in terms of the dark noise when PMT was cooled to 5°C, 10°C and 15°C, and the results are shown in Figure 3.

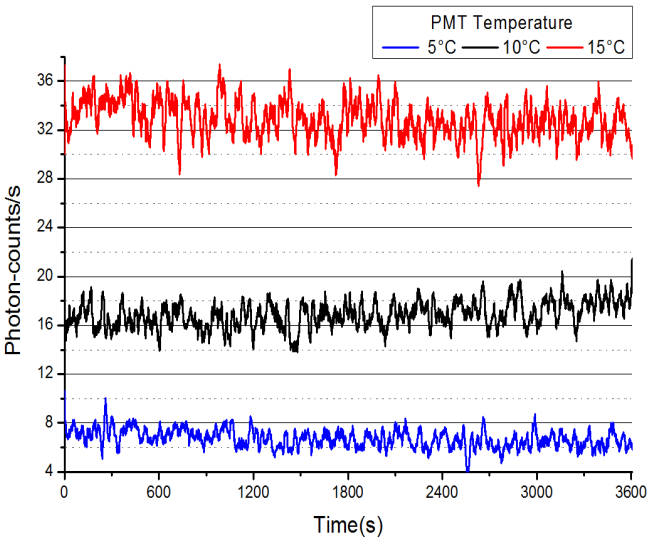

Figure 3. Chamber's dark-noise photon-counting measurements for 1-hour at three different temperatures.

Even for 15°C the dark-noise may be considered appropriated for some biological tests, as those as those using seeds as biological samples, or tests involving DL measurements where the level of UWPE is greater than those of spontaneous nature, since it is comprised of the sum of two signals, that is, the spontaneous and stimulated emissions [18].

The photon counts statistics can be seen in Figure 4, for the 3 temperatures. It is possible to see that for a temperature of 15°C the dark-noise is about 14 counts/s, very wide and little resemblance to Gaussian function. On the other hand, for a temperature of 10°C the dark-noise is about 17counts/s, which was considered the best choice for the PMT operation. At lower temperatures, as for 5°C, water condensation may occur inside the PMT and it may be a serious risk for its integrity.

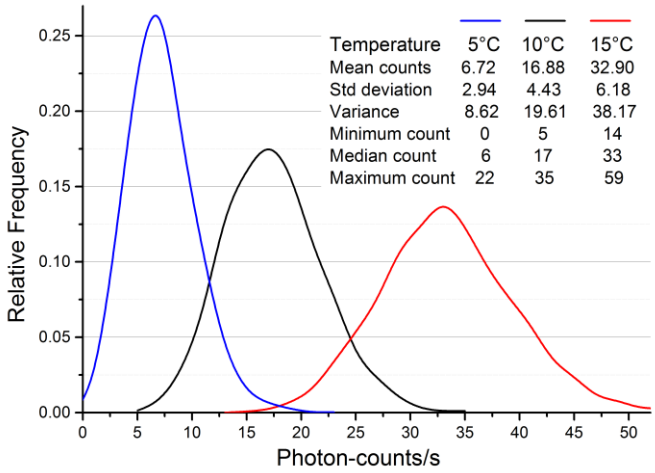

Figure 4. Photon-counting statistics from the dark-noise measurement tests.

B. Measurement of Ultra-Weak Luminescence of Standard Escherichia coli Cultures

Three *Escherichia coli* culture samples prepared as explained before, growing inside the dark chamber for a period of 24 hours presented an emission profile that reflects its three microorganisms growing phases, known as lag, log or exponential and stationary, as shown in Figure 5.

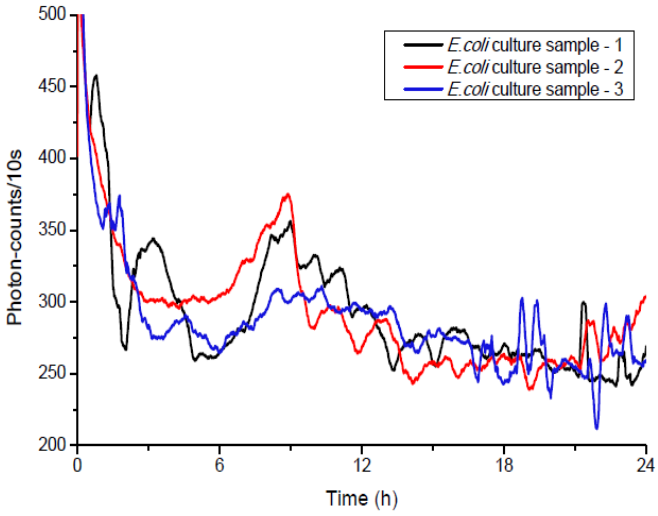

Figure 5. Photon-counting from three *Escherichia coli* tests.

In the lag phase the *Escherichia coli* culture are enzymatically adapting to the substrate, and there is no substantial growth of the culture, this phase appears in the plots of Figure 5 up to the 6th hour. Notice that for all plots of Figure 5, a strong decrease in intensity up to the third hour is present, and it is related to the delayed luminescence (DL) produced by the luminous excitation of the samples by ambient light before entering the dark chamber [22, 23].

After the lag phase the culture enters in the log phase and it starts to grow constantly, as the nutrients are in excess to the bacteria population, what can be seen in Figure 5, from the 6th hour up to the 9th hour.

The stationary phase starts when growing and death rates remain constant, and the nutrients starts to lack. As a result, the culture starts to decline, from the 9th hour up to the end of the tests.

In addition, it is important to note that there is a strong drop of initial photon-countings in the period up to the 6th hour, being this fact related to the delayed luminescence (DL) [22, 23] produced by the light excitation of the sample before it was placed inside the dark chamber.

C. Measurement of Ultra-Weak Luminescence of River's Water Escherichia coli

The tests using the river's water samples, prepared as explained previously, resulted UWPE profiles with some similarity with the emission profiles of standard*Escherichia coli* culture samples, as can be seen in Figure 6.

As mentioned for the tests of Figure 5, in Figure 6 the delayed luminescence produced by the excitation of light in the sample before it was left inside the dark chamber is also verified in all tests. It is possible to verify that the growth kinetics is evident from the 9th until the end of the tests in the three tests performed, what can be clearly correlated to the standard *Escherichia coli* culture tests.

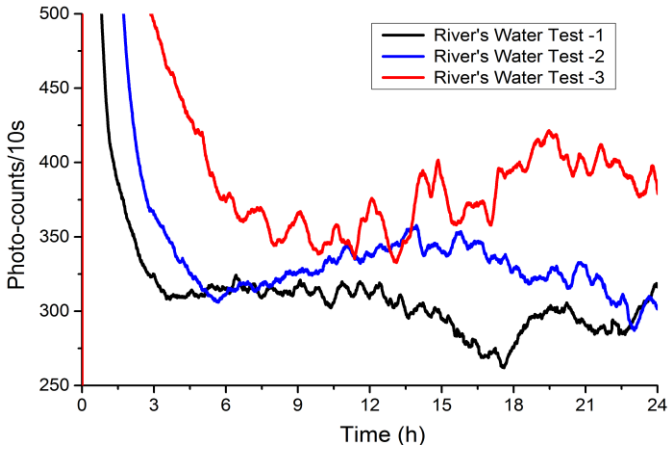

Figure 6. Photon-counting from River's Water tests.

The comparatvive results showed that it is possible to verify the growth kinetics of the coliform group using the instrumentation designed and implemented as explained for the *Escherichia coli* detection from contaminated water samples. The detection may be considered effective, since not only the growth kinetics indicates that, but also the fact that the EC culture medium being specific for the coliform group, the bacterial discrimination is assured.

IV. Conclusions

An ultra-weak measurement system applied to sanitary control was described here in details. The designed and implemented instrumentation was presented regarding its resources and capabilities. The system performance was evaluated in terms of dark noise for three different PMT temperatures, and tests using *Escherichia coli* culture samples. Regarding the proposed application, the aim was to evaluate the system capability in detecting bacterial contamination in water samples collected from a river in a metropolitan area in Brazil.

The UWPE measurements from both samples, that is, standard *Escherichia coli* culture samples and river's water samples, using the implemented measurement system showed that the system can track the three bacteria growth phase.

The UWPE data acquired from the standard *Escherichia coli* culture samples and river's water culture samples, represented by its temporal patterns, pointed out to its potential application in sanitary control of water samples in order to detect the presence of microorganisms of the coliform group.

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