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Experimental study on photocount statistics of the ultraweak photon emission from some living organisms

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Abstract. The hypothesis that biophotons display a high degree of coherence was tested by measuring photocount statistics (PCS) of the ultraweak photon emission from three living organisms (cucumber seedling, mungbean seedling and soybean rhizobium bacteroids) with a high-sensitivity single-photon counter. For comparison, the same experiments were performed for laser beam, randomized laser beam, chemiluminescence from autoxidation of luminol and the dark counts of the equipment. Photocount distributions, close to Poissonian, were observed for the three tested biological systems but not for the pure chemiluminescence of luminol.

Key words. Ultraweak photon emission; photocount statistics; coherence; biophoton; low-level biological chemiluminescence.

It has been firmly established that all living organisms emit low-intensity luminescence, which differs from the much more efficient bioluminescence of the luciferin-luciferase enzymatic systems. This universal property of organisms is inherently associated with many fundamental processes in biological systems, such as oxidative metabolism[1], cell division and death[2,3], photosynthesis[4], carcinogenesis[5,6] and possibly growth regulation[7]. It is due to the close connections with biochemical processes occurring in living organisms and with the physiological and pathological state of organisms that the ultraweak photon emission may have diagnostic potential in medicine, agriculture and other biosciences. However, it has frequently been asked whether the low-level luminescence is only an adventitious emission of no particular biological significance. The answer seems to be still under debate. A biophysical hypothesis assigning particular importance to the informative and organizational role of the electromagnetic field within living organisms has been proposed by H. Froehlich[8]. As a logical extension, F. A. Popp[9,10] proposed and developed a 'coherence theory', based on the physics of interaction of weak radiation in and with optically dense matter, to explain some essential experimental observations which cannot be interpreted in biochemical terms. These include virtual wavelength-independence of photon emission from living organisms[11,12], the hyperbolic (rather than exponential) decay kinetics of induced luminescence from living cells[13,14] and the higher transparency of dense media (including cell layers) for the photons of biological origin than for light from physical sources[15,16]. The theory suggests that biophoton emission originates from a delocalized coherent electromagnetic field within living tissue and serves as a basis for intercellular communication. Although this biophysical hypothesis still lacks solid experimental support, it opens a new horizon in the holistic interpretation of the ultraweak photon emission and its biological role.

Owing to the low intensity and the broad spectral band of a many-mode field, interferometry cannot be used to study the coherence of the photon emission from living systems. It seems that the only suitable method is the use of photocount statistics (PCS)[17,18]. The PCS method measures the probability, $p(n, \Delta t)$, with which n photocounts are registered by a single-photon sensitive detector in a preset time interval, Δt. The quantum theory[18] shows that a fully coherent field in a stationary state is always subject to a Poissonian photocount distribution:

$$p(n, \Delta t) = \frac{\langle n \rangle^n}{n!} e^{-\langle n \rangle} \quad (1)$$

$$\sigma^2 = \langle n \rangle \quad (1a)$$

where $\langle n \rangle$ is the average counts registered within the preset time interval, and σ^2 is the variance of the distribution. For a single-mode chaotic field, the photocounts follow the geometrical distribution:

$$p(n, \Delta t) = \frac{\langle n \rangle^n}{(1 + \langle n \rangle)^{n+1}} \quad (2)$$

$$\sigma^2 = \langle n \rangle (1 + \langle n \rangle) \quad (2a)$$

For a multi-mode chaotic field, i.e. a chaotic field with $\Delta t \gg \tau$ (τ is the coherence time of the field), the photocounts registered are subject to the following distribution:

$$p(n, \Delta t) = \frac{(n + M - 1)!}{n!(M - 1)!} \left(1 + \frac{M}{\langle n \rangle}\right)^{-n} \left(1 + \frac{\langle n \rangle}{M}\right)^{-M} \quad (3)$$

$$\sigma^2 = \langle n \rangle \left(1 + \frac{\langle n \rangle}{M}\right) \quad (3a)$$

where the number of modes, $M = \Delta t/\tau$, is a measure of the 'degree of freedom' of the chaotic field.

Using the above theoretical criteria, the coherence of ultraweak photon emission from living organisms can be checked experimentally by measuring their PCS. Up to now, we have not seen any such a measurement from other investigators except Popp's group, who measured the PCS of cucumber seedlings[19]. In the present study, we measured the PCS of the ultraweak photon emission from cucumber seedlings, mungbean seedlings and soybean rhizobium bacteroids. For comparison, the PCS measurements were also performed using a He-Ne laser as a fully coherent field, the chaotic field established by randomly scattering the laser beam, and luminescence of a purely chemical origin.

Materials and methods

Mungbean and cucumber seedlings. Seeds of mungbean (*Phaseolus aureus Rexb.*) and cucumber (*Cucumis sativus*) were washed with distilled water, then placed on wet filter paper and incubated at 30 °C for 2 and 3 days respectively. 1 cm-long seedlings were chosen for PCS measurements.

Soybean rhizobium bacteroids. The nodules were harvested from the roots of soybean (Hefong-30), which had been inoculated with Bradyrhizobium japonicum (ATCC 10324), when they flowered after 45 days of growth in a greenhouse. The nodules were rinsed in 0.06 M phosphate buffer (PBS), then crushed with a cold mortar and pestle. Bacteroids were isolated according to Bergersen[20] with minor modifications: after filtration, the suspension of the crushed nodules was centrifuged for 6 min at 2400 × g to remove coarse starch grains, and then at 9000 × g for 6 min to sediment the bacteroids. The final suspension of the rhizobium bacteroids at a concentration of 1.2×10^8 cells/ml was used for experiment.

Chemiluminescence of luminol. 2×10^{-4} M luminol (purchased from Beijing Chemical Company) were dissolved in distilled water at about pH 6.5. The intensity of chemiluminescence due to its autoxidation was adjusted by changing the pH of the solution with NaOH.

Coherent and quasi-single-mode chaotic photon field. A He-Ne laser (2 KV, 5 mA, the Electronics Factory of Qinghua University, Beijing) was used as the coherent photon field. The laser beam was attenuated with a neutral density filter, then introduced into the sample house of the single-photon counter through a quartz-fibre light cable. The quasi-single-mode chaotic photon field was established by passing the attenuated He-Ne laser beam through a fast rotating ground glass. Such a randomized laser beam was then introduced into the sample house for detection with the same light cable as that used for the coherent beam.

PCS measurement. The photons emitted from the living organisms and introduced through the light cable were detected with a laboratory-made computerized high-sensitivity single-photon counter (SPC). To minimize the dark counts, the EMI-9558B photomultiplier, which was used as the photodetector in SPC, was cooled down to −18 °C with circulated cold alcohol and the detector was placed in a dark room. The photocount statistics measurements were performed using a specially designed computer program which registers the photon counts detected by the photodetector in any desired preset time interval, displays the photocount distribution on screen in real-time mode, and calculates and prints out the probability distribution, $p(n, \Delta t)$, and its mean and variance.

In the present study, each measurement consists of 1000 samplings of registered photocounts for a preset time interval. The frequencies with which k photocounts were registered, F(k), were scored. The mean number of registered photocounts, $\langle n \rangle$, and the variance of the distribution, σ^2, were obtained as

$$\langle n \rangle = \sum_{k=0}^{n_{max}} k \cdot F(k) \Big/ \sum_{k=0}^{n_{max}} F(k)$$

$$\sigma^2 = \sum_{k=0}^{n_{max}} (k - \langle n \rangle)^2 \cdot F(k) \Big/ \sum_{k=0}^{n_{max}} F(k)$$

where n_{max} is the largest number of photocounts registered.

Results

Distribution of photocounts in a preset time interval. The distributions of photocounts in three preset time intervals were measured for the photon emission from all three biological systems (soybean rhizobium bacteroids, mungbean and cucumber seedlings), He-Ne laser, the randomized laser beam, the autoxidation of luminol, and for the dark counts for the photomultiplier in the SPC. As an example, figure 1 shows the photocount distributions of the spontaneous luminescence from PBS suspension of rhizobium bacteroids and the chemiluminescence from autoxidation of luminol in alkaline solution at time intervals of 100, 250 and 500 ms. It may be seen from the figure that the photocount distribution of rhizobium bacteroids, representative of the three tested living organisms, seems to be closer to Poissonian distribution (shown as the solid lines in figure 1) than that of the chemiluminescence which represents purely adventitious, nonfunctional events arising in exothermic oxidation. However, it is difficult to judge quantitatively how close a particular distribution is to the Poissonian just by looking at the distribution profile as shown in figure 1.

Agreement of the measured photocount distribution with Poissonian. As a measure of the agreement with Poissonian distribution, Popp suggested taking the value[21]

$$\delta = \frac{\sigma^2 - \langle n \rangle}{\langle n \rangle} \qquad (4)$$

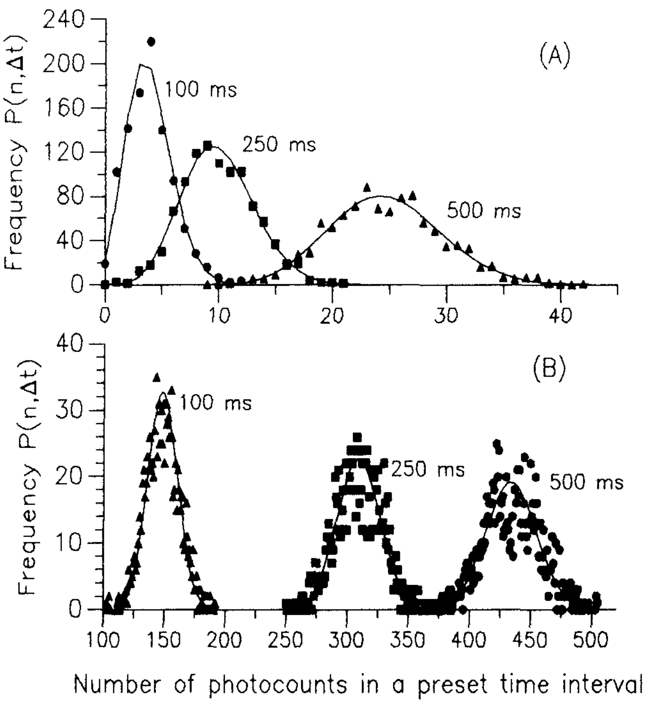

Figure 1. The photocount distributions of the spontaneous photon emission from the soybean rhizobium (ATCC 10324) bacteroids in 2 ml of 0.06 M phosphate buffer (1.2×10^8 cells/ml) (A), and the chemiluminescence from autoxidation of 2×10^{-4} M luminol in alkaline solution (B). Each distribution consists of 1000 samplings corresponding to one of the three present time intervals, 100 ms, 250 ms and 500 ms. The solid lines represent the theoretical Poissonian distributions with the same mean values of each measured distribution.

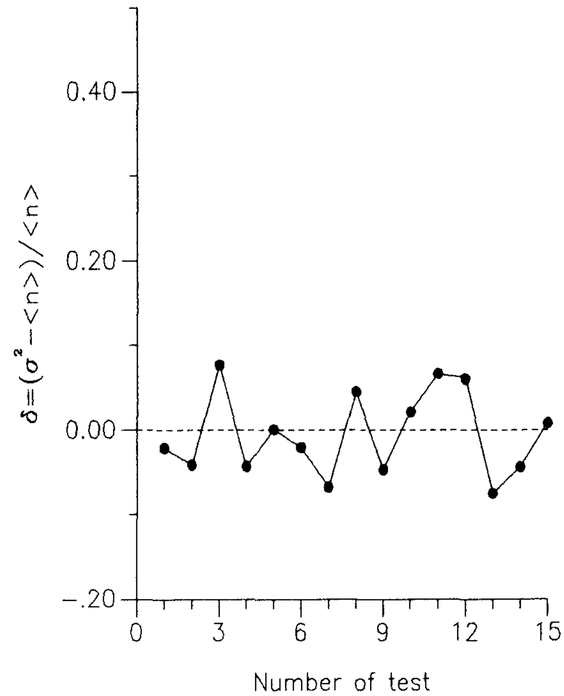

Figure 2. The deviations of 15 computer-generated Poissonian distributions with same average number from the real Poissonian distribution expressed as the δ values (see text). Each computer-generated distribution consists of 1000 stochastic numbers.

Agreement with a Poissonian distribution is expressed as $\delta = 0$, while $\delta > 0$ as a bunching effect indicates a chaotic source, $\delta < 0$ means that antibunching takes place. As an example, the δ-values of 1000 Poissonian stochastic numbers generated by a computer program were calculated for 15 tests and are shown in figure 2. In this program, the stochastic number k was generated by fulfilling the following equation:

$$\prod_{i=0}^{k} r_i > e^{-\langle n \rangle} > \prod_{i=0}^{k+1} r_i \quad (5)$$

where r_0 was set to 1, $r_1, r_2, \ldots, r_k$ were random variables within the range of (0, 1) and generated by the computer program of RND function, and $\langle n \rangle$ is the desired mean of the Poissonian stochastic numbers. Figure 2 shows the δ-values for fifteen computer-generated Poissonian distributions, which fluctuated below and above $\delta = 0$.

The δ-values of ten independent tests were experimentally obtained for He-Ne laser, the randomized laser beam, cucumber seedlings and the noise of the photodetector at -18 °C respectively. Each test consists of 1000 samplings of photocounts registered in the time interval of 100 ms. The results are shown in figure 3. It clearly shows that the δ-values of the photocount distributions

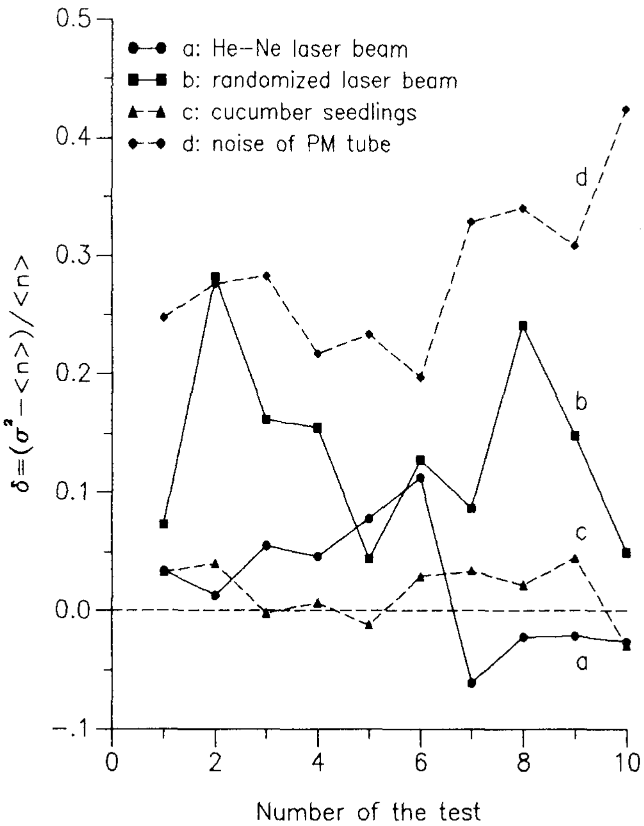

Figure 3. The photocount statistics (PCS) measured for a He-Ne laser beam, random scattered He-Ne laser beam, cucumber seedlings, and the noise of the photomultiplier at -18 °C. Each test consists of 1000 samplings in the time interval of 100 ms.

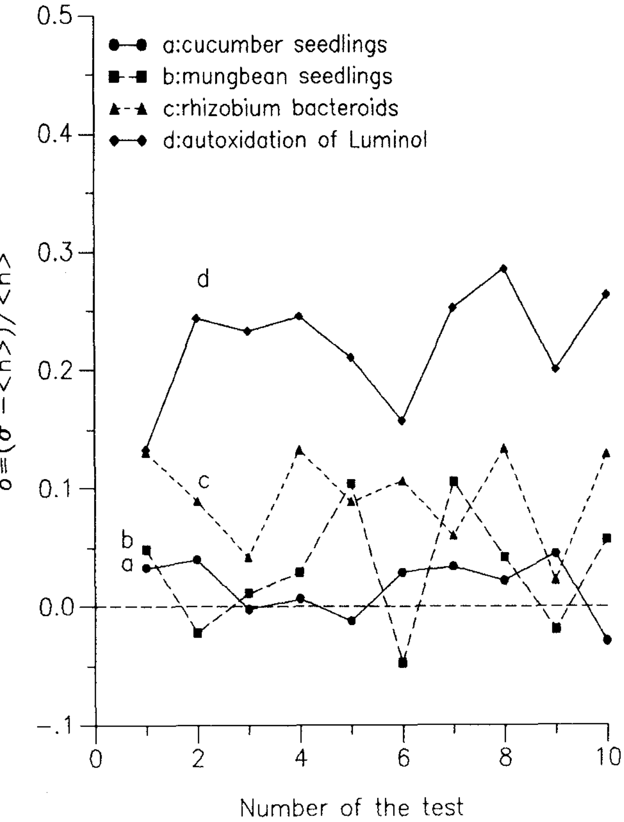

Figure 4. The photocount statistics measured for three living organisms (cucumber seedlings, mungbean seedlings and rhizobium bacteroids) and the chemiluminescence from the autoxidation of 2×10^{-4} M luminol in alkaline solution. Each test consists of 1000 samplings on registered photocounts in the time interval of 100 ms.

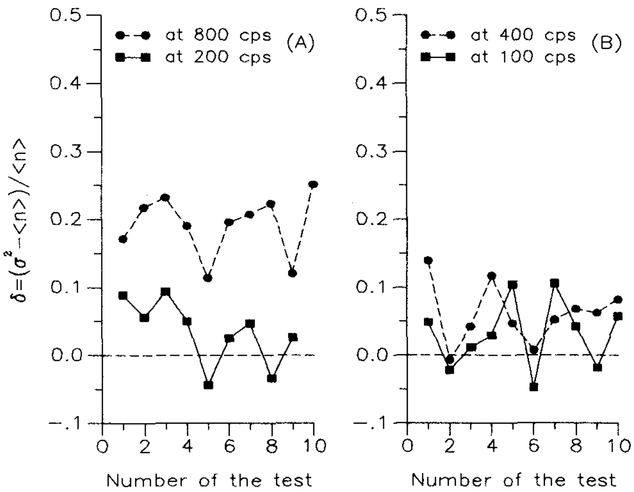

Figure 5. The effect of emission intensity on PCS of the chemiluminescence from autoxidation of luminol (A), and the photon emission from mungbean seedlings (B). The time intervals for registering photocounts are 500 ms in (A) and 100 ms in (B).

for laser beam and for cucumber seedlings are closer to zero, while those of the other two sources are relatively distant from zero. This means that the photocount distribution for laser beam and the living organism are close to Poissonian but the distribution for a randomized laser beam of chaotic nature and the thermal emission from the cathode of photomultiplier are relatively far away. Similar to figure 3, the δ-values of ten independent tests for three biological systems and the chemiluminescence from the autoxidation of luminol are shown in figure 4, which clearly shows that the photocount distributions of the three living organisms are much closer to Poissonian distribution than that of the pure chemiluminescence.

Effect of emission intensity on PCS. The intensity of a light source must affect the mean value of the measured photocount distribution and changes the deviation of a non-Poissonian distribution from Poissonian (see equations 2a, 3a and 4). The photocount distribution of the chemiluminescence in autoxidation of luminol at a count rate of 200 cps and 800 cps and the distribution of the photon emission from mungbean seedlings at the count rate of 100 cps and 400 cps were measured. The δ-values of the measured distributions are shown in figure 5. It can clearly be seen that as the intensity increased, the δ-values for the chemiluminescence became larger, but did not change significantly for mungbean seedlings. The difference in the intensity effect between the pure chemiluminescence and the photon emission from mungbean seedlings indicates that these two light sources may have different degrees of coherence in nature.

Effect of sampling time interval on PCS. Increase of the sampling time interval, Δt, will result in increase of the mean value of photocounts detected in Δt, which must change the δ value of a non-Poissonian distribution. Therefore, we measured the photocount distributions of the photon emission from the three living organisms and the noise of the photomultiplier in SPC as an ideal chaotic source with different sampling time intervals. The δ-values of each measured distribution were calculated and shown in figure 6. As expected, the chaotic noise of the photomultiplier gives rise to increasing δ-values with increasing Δt. However, increasing Δt (from 100 ms to 500 ms) does not seem to increase significantly the δ-values for the photocount distribution of the three biological systems.

Discussion

In the past decade, improvements in the single-photon counting technique and new methodological approaches have allowed scientists to derive more precise phenomenological pictures of parameters and processes involved in the low-intensity photon emission from living organisms. Conceptual developments have resulted in two basic hypotheses: (1) the biochemical one, claiming that biological chemiluminescence is only an adventitious emission from purely chemical reactions of no

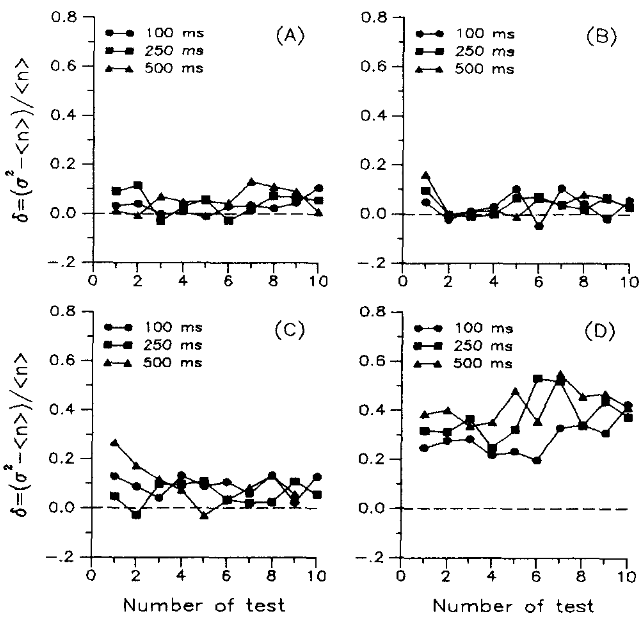

Figure 6. The effect of the sampling time interval on photocount statistics of the spontaneous photon emission from cucumber seedlings (A), mungbean seedlings (B), rhizobium bacteroids (C), and the noise of photomultiplier at −18 °C in SPC (D).

particular biological significance, and (2) the biophysical hypothesis, assigning particular importance to the informative and organizational role of the electromagnetic field within living organisms. Experimental evidence accumulated so far leaves no doubt as to the validity of the biochemical interpretation of the chemi-excitation and its association with metabolism in the biological systems. However, we still need more experimental data to verify the biophysical hypothesis, which has the coherence theory as its core. The aim of the present study is to provide more data, rather than evidence, for understanding the possible coherence of the photon emission from living organisms. The results of this study show that the photocount distributions for the three living organisms are truly close to Poissonian distribution, and do differ from the distributions obtained for the quasi-single-mode chaotic field established by randomizing a laser beam, for the ideal chaotic noise of a physical device (i.e. photomultiplier), as well as for the pure chemiluminescence from the autoxidation of luminol. The difference between the three living organisms and the chemiluminescence from the autoxidation of luminol in photocount statistics may be particularly interesting. The difference in change of sampling time interval and emission intensity between the three biological systems and the chaotic noise of the photodetector or the chemiluminescence from luminol autoxidation may further imply that the photon emission from living organisms is not an adventitious emission from pure chemi-excitation. However, the existence of Poissonian distribution is only a necessary, but not a sufficient condition for coherence. As may be seen from equations 3 and 3a, for a multi-mode chaotic field, or more generally for a chaotic field with a much longer sampling time interval than the coherence time, the distribution will approach the Poissonian because of $M \gg \langle n \rangle$. In this study, we also measured the photocount distribution of tungsten lamp and observed a good agreement with a Poissonian one. We face a serious difficulty: for a stationary system one cannot distinguish a coherent field from a complete chaotic field, as long as the coherence time of the chaotic system is significantly shorter than the measurement time interval.

It should also be pointed out that the photocount distributions of three living organisms are not completely in agreement with Poissonian distributions, because the δ-values obtained are rare below zero. This is particularly true for the photon emission from rhizobium bacteroids which was relatively low in intensity. This could be, at least partly, due to the unavoidable chaotic noise of the equipment, which was about 15 cps on average throughout the investigation and sometimes covered one tenth or even one third of the registered counts, superimposed on the photocounts deriving from the organisms. The other possibility is that the photon emission from biological systems is actually only partly coherent.

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