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Super-high sensitivity systems for detection and spectral analysis of ultraweak photon emission from biological cells and tissues

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Summary. In this paper we summarize and discuss the modern technology and systems, studied and established by our research group, for performing the detection and special analysis incorporated with the super-high sensitivity photon counting method for the study of ultraweak photon emission; for example, extra-weak bioluminescence and chemiluminescence from living cells and tissues, closely related to biochemical and biophysical processes and activities. An excellent sensitivity of the basic photon counting system, making it possible to achieve count rates in the very low range of one photoelectron per second to one per minute, allowed us to carry out in vivo as well as in vitro measurements, and analyses of ultraweak bioluminescence and chemiluminescence. Recent results concerning ultraweak photon emission from blood samples and organ homogenates of rats are presented and reviewed as one of the interesting and valuable applications of our modern technology for studying ultraweak cell and tissue radiation.
Key words. Ultraweak photon emission; bioluminescence; chemiluminescence; super-high sensitivity photon counting technique; photon counting spectral analyzer system; biological cells and tissues; rat blood; rat organ homogenates.

Introduction

Recently there has been a great deal of interest in and considerable need for measurement of light intensities and their spectral properties at extremely low levels, with the advancement of optical electronics and the variety of its applications. Work is being done on the measurement of photons in the optical region under extreme conditions, and research using these developments for extracting information carried in ultraweak light from various kinds of faint sources, either emitters or scatterers, which release only a small number of photons is attractive and important. This technology can also be expected to provide new discoveries and valuable knowledge about ultraweak photon emission such as extra-weak bioluminescence and chemiluminescence, associated with biochemical and biophysical processes, from biological cells and tissues.

With this aim, we have designed and developed a super-high sensitivity photon counting system employing specially selected low-noise photomultipliers. By optimizing systematically its operating conditions and by shielding the photomultiplier and electronics completely from surrounding noise sources, an excellent high sensitivity was realized, which made it possible to have count rates in the extremely low range of one photoelectron per second to about one per minute. Furthermore, the systematic study of the method for analyzing the spectral distribution of extra-weak photon emission enabled us to design and construct successfully a new type of very high sensitivity spectrometer, called the filter spectral analyzer system, which incorporated both the ultra-high sensitivity photon counting technique and a series of colored glass filters controlled by a microcomputer.

As a fascinating potential application of these super-high sensitivity photon counting and spectral analyzing systems, we have pursued the measurement and analysis of extremely weak bioluminescence and chemiluminescence from various biological and chemical systems, together with their ultraweak spectra in the near ultraviolet and visible regions. The systems studied included living tissues such as malignant tumors and various organs, blood and urine samples, enzymatic reactions and biochemical processes involving free radicals, lipid peroxidation, oxidative deterioration of oils, fats and various kinds of foods, and so on.

This paper is devoted to describing and summarizing the principle and the performance of these super-high sensitivity intensity detection and spectral analysis systems incorporating the photon counting technique, which have been constructed and operated by our Biophoton research group. Furthermore, recent results of measurements and analyses of ultraweak photon emission, from blood samples and organ homogenates of rats, are described. This emission could be associated with the reactive mechanism of in vivo lipid peroxidation. Such measurements are discussed as one of the valuable and attractive applications of our modern technology for ultraweak cell and tissue radiation.

Super-high sensitivity photon counting system

Three kinds of photoelectric methods to detect weak light signals are at present being employed in the spectral region accessible to a photomultiplier (PM). The first and second ones are DC and AC methods, based on the analog scheme, where the light intensity information is extracted out of the DC component, and the AC component or associated shot noise in the PM output, respectively. The third one is customarily called the single photoelectron counting (SPC) or, simply, the photon counting method [18] where contributions from photoelectrons are resolved in time so that the signals in the form of electron pulses are detected by means of a pulse-counting electronic system. The phase-sensitive technique can be utilized in each method to improve the sensitivity and the stability. They are called SDC, SAC and SSPC methods, respectively, where the first S stands for 'synchronous'. Comparative studies performed experimentally and theoretically on the detection sensitivity of these six schemes have demonstrated that the SPC and SSPC methods are better than the other methods for the measurement of ultraweak light signals [5, 6, 23]; this fact is well recognized nowadays.

Thus the photon counting technique has been developed practically in this decade and is widely utilized to detect very weak light intensities in the wavelength region from near ultraviolet to near infrared in conjunction with highly sensitive PMs. In general, the optimum operational conditions of the photon counting method are achieved by maximizing the figure of merit M_f defined by

$$M_f = \eta(\lambda)\, F(v_d)\, A_c \tag{1}$$

as described by the present author [8]. Here $\eta(\lambda)$ is the quantum efficiency of the photocathode, and A_c is the effective photocathode area which is determined by the acceptance angle, the distance between the measured sample and the photocathode, and the actual photocathode area. $F(v_d)$ is expressed by

$$F(v_d) = D_S(v_d)\sqrt{T}/\sqrt{\langle n_N\rangle D_N(v_d)} \tag{2}$$

where v_d is a selected pulse-height discriminator voltage, $\langle n_N\rangle$ is the average count rate of the noise pulse and T is the detection time. $D_S(v_d)$ and $D_N(v_d)$ are the probabilities of occurrence of counting pulses, above the discriminator voltage v_d, caused by single photoelectrons and by noise current electrons, respectively.

From equations (1) and (2), one can understand that it is essential to select a PM which possesses as large a value of $D_S(v_d)/\sqrt{D_N(v_d)}$ as is possible, as well as a large photocathode area with low noise count rate, to give a high value of $A_c/\sqrt{\langle n_N\rangle}$. The maximum value of $D_S(v_d)/\sqrt{D_N(v_d)}$ is expected for a PM which has distinctly dif-

ferent pulse-height distributions for photoelectron and noise electron pulses. Although there exist PMs designed for photon counting which have low noise count rate, the effective photocathode area is usually small ($\lesssim 1$ cm^2) in order to achieve the desired count rate. We should mention here that it is inherently important to select a specifically low noise count-rate PM with a photocathode of large diameter for the detection of extremely weak photon emission, such as ultraweak bioluminescence from biological cells and tissues and ultraweak chemiluminescence associated with various sorts of chemical and biological processes. Moreover, its pulse-height distribution for photoelectron pulses, which usually exhibits a large bump corresponding to the single photoelectron event, is required to be far from that of noise electron pulses which show almost an exponential curve.

On the basis of these studies, we have designed and constructed a super-high sensitivity photon counting system suitable for biochemical and biophysical measurements as well as for biomedical applications [8, 9]. Figure 1 shows a schematic diagram of this system. Specially selected low noise PMs such as HTV R 878, R 550, R 375 with a 50-mm diameter photocathode, manufactured by Hamamatsu Photonics Corp., Japan, are used. The photocathode is cooled by a thermoelectric cooler to minimize noise. The optical system between the photocathode and the sample room is so designed as to widen the effective solid angle for increasing the overall light collecting efficiency. By optimizing systematically the operating conditions and by shielding the PM and electronics completely from surrounding noise sources, an excellent high sensitivity was realized.

The intensity of the detected ultraweak photon emission is indicated by the count rate of a single photoelectron pulse with a detection time which can be as long as several minutes, and is recorded by a printer and a recorder. It is also possible to link this system with a microcomputer for data processing and control as well as for display of necessary data. The sample room can hold a sample cell of 50 mm in diameter and a few mm to 20 mm in height. The sample to be measured can be either a liquid, a massive solid or a powder, or even a gas, and can be kept at any temperature between room temperature and 200 °C by the controlled heater. The atmosphere in the sample cell can also be replaced with an other gas by the use of an air pump.

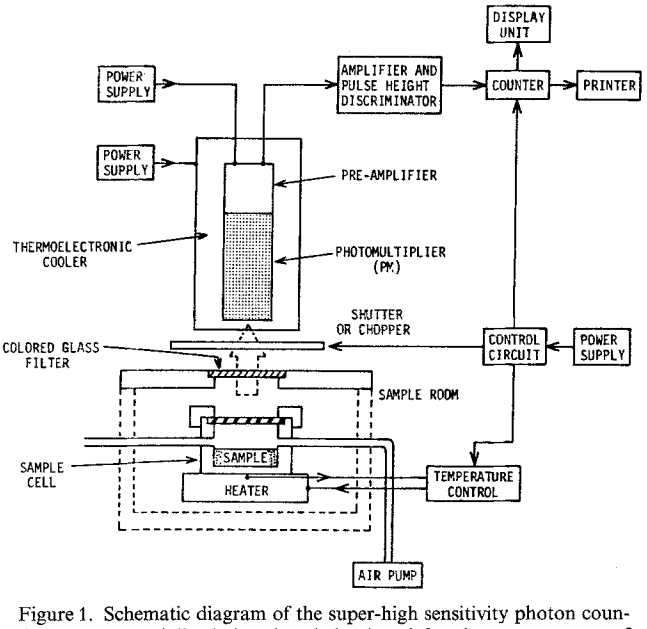

Figure 1. Schematic diagram of the super-high sensitivity photon counting system specially designed and developed for the measurement of ultraweak photon emission involved in biochemical and biophysical processes along with biomedical applications.

A highly sensitive photon counting system named 'Chemiluminescence Analyzer OX-7', which has been developed under a contract with the Research Development Corporation of Japan (JRDC) and is now commercially available from Tohoku Electronic Industrial Co., Ltd., Sendai, Japan, has an excellent sensitivity, making it possible to achieve a count rate as low as one photoelectron per second or better. The series includes several models, such as one for performing in vivo measurements on live specimens or live test animals employing an optical box connected to the Chemiluminescence Analyzer OX-7 with an optical fiber of 10 mm in diameter. Shelves on which to place specimens or test animals, as well as large samples such as those used for in vitro experiments, can be moved up and down to adjust the distance between the specimen and the optical fiber head. The position of the optical fiber head, which is attached to a stainless steel bar inside the optical box, can be controlled precisely by sliding the bar horizontally towards front and rear, and by shifting the head right and left along the bar. The size of this optical box is approximately 100 cm in width, 85 cm in length and 104 cm in height, and four gas joints are installed for substituting various gases inside the box when it is necessary.

A more sophisticated photon counting system fully operated by a microcomputer (NEC PC-9801), named 'Chemiluminescence Analyzer CLA-700', was quite recently developed by Tohoku Electronic Industrial Co., Ltd. Its detection sensitivity is about one order of magnitude higher than the conventional OX-7 system as shown in figure 1. Such an extremely high sensitivity could be achieved by very carefully maximizing the figure of merit M_f given by equation (1), especially by adjusting the value of $A_c/\sqrt{\langle n_N \rangle}$ of each of the specially selected PMs for the photon counting, and by complete electronic shielding of the system.

High-sensitivity photon counting spectral analyzer system

In parallel to the development of a super-high sensitivity photon counting system for detecting ultraweak photon emission from biological cells and tissues, and that involved in various kinds of chemical and biological reactions, we have carried out a study in order to establish an

appropriate method for the spectral analysis of this ultraweak photon emission, in conjunction with the photon counting technique[6, 7]. In this study, we were primarily interested in the spectral measurement of ultraweak bioluminescence and chemiluminescence, which are usually accompanied by the following main features:

a) Their intensity is mostly very weak; the total power is of the order of 10^{-15} W or less.
b) Most spectra exhibit broad band emissions in the wavelength range between ultraviolet and near infrared. Therefore, high resolution is not required.
c) A large emitting area is mostly preferable for the ultralow level photon detection.
d) Intensity often changes slowly with time.

For a measurement system suitable for this particular kind of spectral analysis, the following conditions should be satisfied:

1) Optical efficiency of the whole system should be high, so as to minimize the total optical loss as far as possible. 2) The entrance aperture should be large, and independent of the resolving power. 3) A large solid angle of the entrance aperture is required. 4) The system must have good reproducibility of measurement, and a simple structure for easy operation.
Among these requirements, it is of primary importance to achieve a minimum total loss in the optical system.
In order to satisfy these conditions in practice, we decided to employ a set of colored glass filters as a wavelength selector which possesses different sharp short-wavelength cutoffs, as shown schematically in figure 2 a, instead of a combination of a wavelength dispersion element such as a grating or a prism, and a slit. It was confirmed experimentally that the colored glass filter generally has a wider acceptance angle than the interference filter without appreciable shifts of the transmission wavelengths. Also it is well known that the transmission of a colored glass filter is usually much better than that of an interference filter and a wavelength dispersion element.

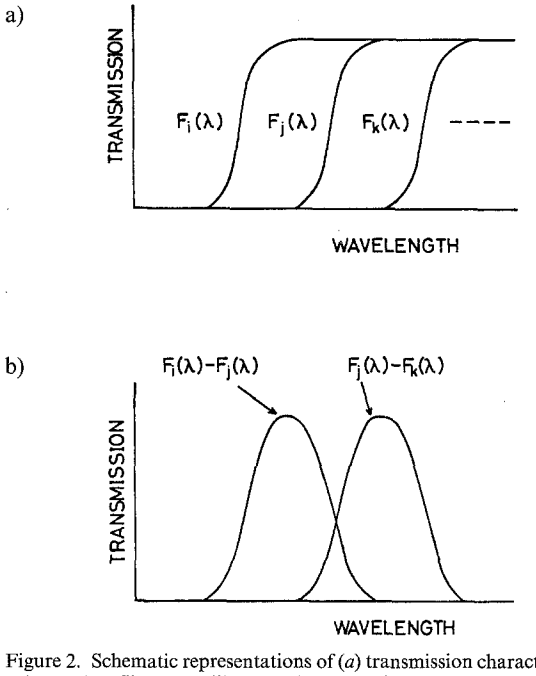

Figure 2. Schematic representations of (*a*) transmission characteristics of colored glass filters and (*b*) spectral window defined by the subtraction of two transmission curves corresponding to the successive colored glass filters.

Figure 3 indicates the block diagram of the system developed on the basis of this principle, called a filter spectral analyzer system (FISAS), including the data processor. It was devised for the spectral analysis of an extremely weak bioluminescence and chemiluminescence originating from a large emitting area. Some modifications appropriate for any other types of ultraweak photon emission source can be readily performed. For routine measurement, the light-emitting sample is placed in a sample cell made of quartz which is thermoelectrically controlled at a suitable temperature for the measurement. The emitted photon is collected effectively onto the photocathode of a specially selected low noise PM by means of an ellipsoidal light reflector. The PM is thermoelectrically maintained at a lower temperature of $-20\,^{\circ}C$ in order to reduce the dark current.
The spectral analysis of ultraweak light signals is accomplished with the successive insertion of colored glass filters arranged on a rotating disc into the optical path between the reflector and the PM. 27 colored filters commercially available from Toshiba Corp., Japan (IA-1017 A) are utilized to cover the total wavelength region between 275 and 670 m, with the transmission ranging from 45% to 60%. The insertion is performed automatically by means of the filter drive controller to cover the required range of the spectrum. The optical signals, transmitted through the individual colored filters, are converted into single photoelectron pulses by the PM to be detected by either the SPC or SSPC method by selecting the mode of the reversible counter with an appropriate detection time. The chopper and the phase shifter depicted in figure 3 are used for the SSPC scheme. This filter spectral analyzer system is 160 cm in width, 60 cm in length, and 110 cm in height, including an electric reversible counter, a digital printer and a regulated DC power supply.
The spectral measurement is carried out by computing the count rate for the individual spectral window defined by the subtraction of two transmission curves with different sharp short-wavelength cutoffs corresponding to the successive colored glass filters, as illustrated schematically in figure 2 b, followed by the calibration of the spectral sensitivity of the photocathode. Accordingly, the average optical intensity P_{ij} between the ith and jth successive colored glass filters is expressed by

$$P_{ij} = e(N_i - N_j) \int S_c(\lambda) [F_i(\lambda) - F_j(\lambda)] \, d\lambda \qquad (3)$$

where $N_i - N_j$ is the subtraction of the count rate and

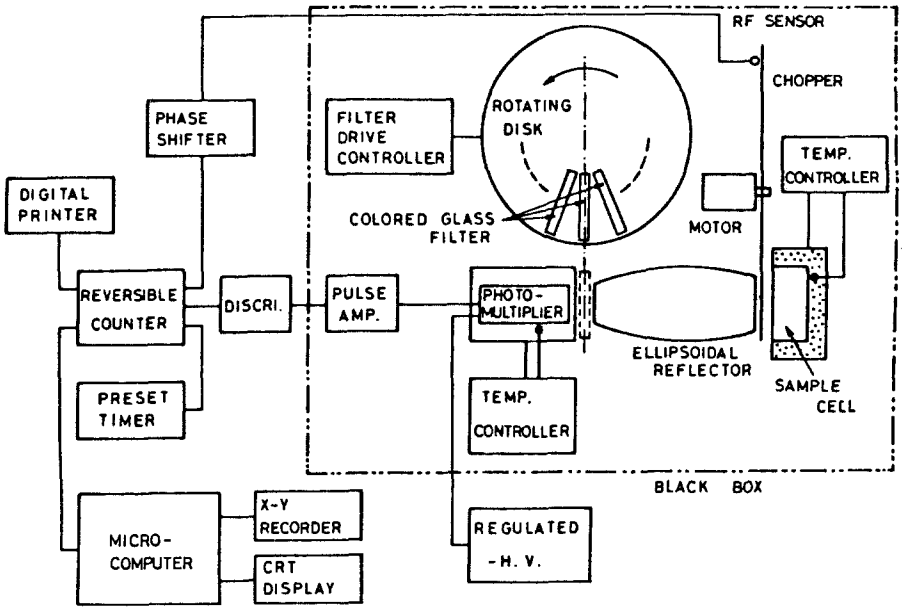

Figure 3. Block diagram of the filter spectral analyzer system (FISAS) controlled and processed by a microcomputer, for spectral measurements of ultraweak photon emission such as associated with biochemical and biophysical processes and activities, incorporated with the very high sensitivity photon counting technique.

$F_i(\lambda) - F_j(\lambda)$ is the subtraction of the transmission curve of the ith and jth successive filters, $S_c(\lambda) = \lambda\eta(\lambda)/hce$ expresses the spectral sensitivity of the PM's photocathode, and e is the electronic charge, respectively. This data processing is handled by an on-line microcomputer to give the spectral information of ultraweak light signals in the form of a histogram, which can be convoluted by a Lorentzian function representing well the shape of the spectral window, i.e. $F_i(\lambda) - F_j(\lambda)$, when this is necessary.

Figure 4 shows the flow chart for operating this photon counting spectral analysis system, by carefully taking into account the possibility of slowly changing intensity characteristics of the ultraweak bioluminescence and chemiluminescence with time, as pointed out previously. This chart explains more practically the above procedures, in connection with the successive processes for extracting spectral information schematically illustrated on the right-hand side of this figure. The spectral resolution of this system, which depends primarily on the geometry of the individual spectral window, is estimated to be 30–50 nm for the wavelength region 275–450 nm and 20–25 nm for the wavelength region 450–670 nm.

For experimental tests to evaluate the performance and the operational characteristics of this system, we made the spectral analysis of considerably attenuated light sources with well-known spectral distributions such as a low pressure mercury lamp, and a luminol and hydrogen peroxide system in alkaline solution, which produce a reaction accompanied by the chemiluminescence of which the total spectral power is of the order of 10^{-13}–10^{-16} W. It was confirmed that the filter spectral analyzer system is superior by about 2 orders of magnitude to the conventional grating spectrometer of f/3.5 incorpo-

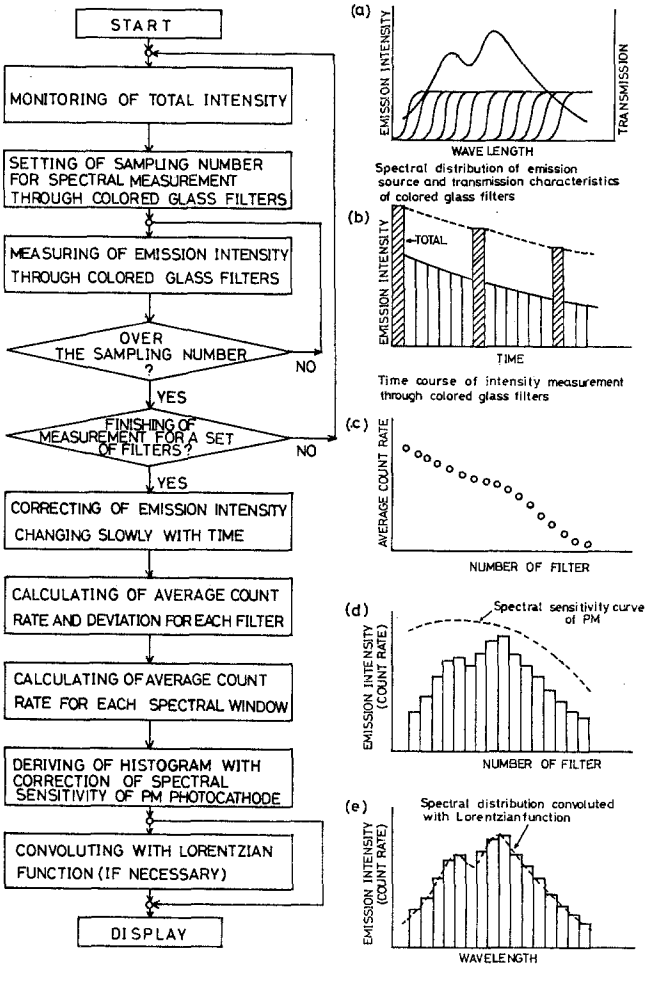

Figure 4. Flow chart for the operation of the filter spectral analyzer system (FISAS) incorporated with the very high sensitivity photon counting method, and schematic illustrations of the successive process for extracting spectral information according to this flow chart.

rating a light collecting lens with regard to the sensitivity, but with sacrifice of the spectral resolution[6, 7].

Figure 5 illustrates the experimental comparison between the spectroscopic data for chemiluminescence in the luminol-hydrogen peroxide system obtained by our spectral analyzer system and the conventional grating spectrometer. In the upper part of the figure, both of the relative distributions of the spectra are shown; one is measured at the total input power of 10^{-15} W by the former with the average resolution of 30 nm, and the other is measured at about 10^{-13} W by the latter with the spectral resolution fixed at 30 nm. The general tendency towards agreement between them is readily seen, although the detection times of the two instruments were set at 8 s and 10 s, respectively. For a reference for the spectral intensity, another two traces of the measured spectrum at the total power of 3×10^{-14} and 10^{-14} W by the grating spectrometer are also shown.

These results demonstrate that this new type of spectral analyzer system has a very high sensitivity and excellent capability for the spectral measurement of ultraweak bioluminescence and chemiluminescence.

Measurement and analysis of ultraweak photon emission from blood and organ homogenates from rats

As an interesting potential field for the application of these super-highly sensitive intensity detection and spectral analysis systems incorporating the photon counting technique, we have performed the measurement of ultraweak bioluminescence and chemiluminescence and the analysis of their spectra in the near ultraviolet and visible regions, from various biological and chemical systems. The systems investigated included living tissues, like malignant tumors, organs[9, 23] and blood[8–10, 16, 29, 30], and also urine[11], enzymatic reactions and biochemical processes involving free radicals[1, 13, 20, 25, 31], lipid peroxidations[9, 15, 17, 19], and oxidative deterioration of oils, fats and foods[9, 21, 26]. In this section, some of the results are presented. Using rat tissues, a study was made of ultraweak photon emission associated with the process of lipid peroxidation, which is thought to have a close relationship with tissue damage and aging, and to be the cause of various disorders and diseases of living cells and tissues, as is well known at the present time.

Figure 6 shows an example of the ultraweak photon emission intensity measured in rat blood at different ages (in months)[9]. The TBA (thiobarbituric acid) value and the DPPH (α, α-diphenyl-β-picryl hydrazyl) value were also measured for comparison. The TBA assay is one of the most commonly used methods for the quantitative measurement of lipid hydroperoxides in living tissues, and is also usually utilized in medicine for evaluating the degree of aging. The value of DPPH is understood to provide a measure of the antioxidative activity of living tissues and systems; the smaller the value, the larger the activity. The number of rats employed is given by n, and the data marked O_2 are those obtained under a defined oxygen gas flow, for the quantitative measurement. Columns labeled 'tca' represent data from rat blood samples treated with trichloroacetic acid.

There is a marked difference of about 4–5 times in photon emission intensity of the blood between 2-month-old and 11-month-old rats. Similarly, the comparisons of TBA and DPPH values between 3-month-old and 12-month-old rats also reveal a tendency for both the values to increase with aging. However, these differences were not so evident as in the case of ultraweak photon emission intensity measured by our very high sensitivity photon counting system.

It is generally known that the exposure of living systems and tissues to radioactive radiation and ultraviolet light

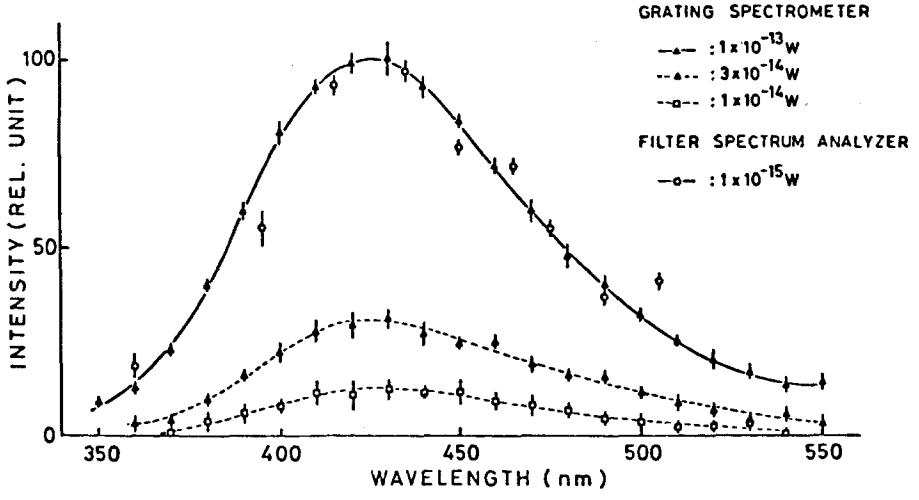

Figure 5. Measured spectral distribution of spontaneous chemiluminescence in luminol-hydrogen peroxide system in alkaline solution by the use of the filter spectral analyzer system (FISAS) and a conventional grating spectrometer. The relative distribution was compared between the spectrum measured at the total intensity of 10^{-15} W for the former, and that at 10^{-13} W for the later. Another two data corresponding to the total intensity of 3×10^{-14} and 10^{-14} W are shown on the same scale with that for 10^{-13} W obtained by the grating spectrometer.

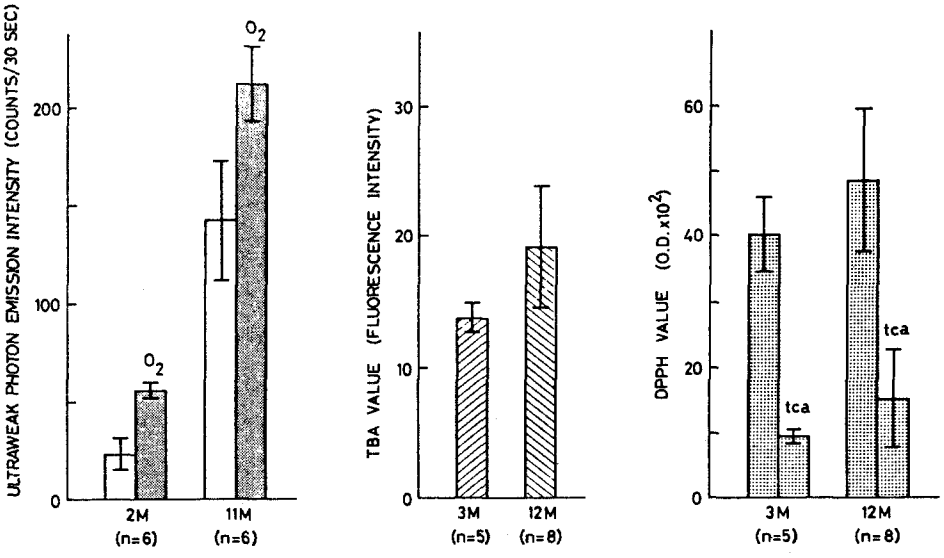

Figure 6. Comparison of measured results of the ultraweak photon emission intensity, the TBA value and the DPPH value of rat blood samples with different ages (in months).

results in the enhancement of generation of free radicals, and this lowers their antioxidative activity by accelerating the lipid hydroperoxidation inside them. In order to verify this fact, we irradiated male rats weighing about 200 g with light from a 15 W ultraviolet lamp for 90 h at a distance of 40 cm. Comparisons of ultraweak photon emission intensities of various tissues with and without ultraviolet irradiation were made. The table summarizes the measured results for blood plasma and homogenates of internal organs, such as heart, lung, liver and kidney, along with the TBA and DPPH values[9]. One can recognize that all results demonstrate clearly marked differences in photon emission intensity and similar changes in both the TBA and DPPH values. As a consequence, the evidence in this table should support the usefulness of the detection of ultraweak photon emission as a monitoring method for tissue lipid peroxidation associated with ultraviolet radiation.

While rat blood and organ homogenates were found to emit ultraweak spontaneous chemiluminescence which is enhanced by the progression of in vivo lipid peroxidation[9, 14], Boveris et al.[2] also indicated that in situ perfused rat livers produce an increase of very weak spontaneous emission by infusion of exogenous hydroperoxides. Tocopherol deficiency has been reported by several workers as one of the causes of an increase of lipid peroxidation in animal tissues[3, 4, 22]. Experimental studies were therefore performed on the characteristics of ultraweak chemiluminescence which is spontaneously emitted from tocopherol-deficient rat tissues such as organ homogenates and blood[9, 16, 17].

Comparison of the ultraweak photon emission intensity, the TBA value and the DPPH value of blood plasma and homogenates of internal organs of rats without and with ultraviolet irradiation (♀ rats, ~ 200 g)

	UV irradiation	Emission intensity (counts/s)	TBA value (O.D. × 10^2)	DPPH value (O.D. × 10^2)
Plasma	without	8	3.1	4.4
	with	141	3.4	29.5
Heart	without	19	3.4	2.7
	with	90	8.5	12.6
Lung	without	34	4.0	4.9
	with	238	20.0	11.3
Liver	without	10	4.8	2.5
	with	126	29.5	10.9
Kidney	without	64	4.7	2.9
	with	87	21.0	10.1

TBA thiobarbituric acid; DPPH, α,α-diphenyl-β-picryl hydrazyl.

In figure 7, curves A and B show temporal changes of ultraweak photon emission from liver homogenates prepared from tocopherol-deficient rats[16]. These two measurements were carried out using liver samples of 500 mg, homogenized with 4.5 ml of physiological saline, or with 4.5 ml of 0.9% NaCl – D_2O. For this study, male Wistar rats weighing about 100 g were fed either commercial pellet rations containing 15 mg of tocopherol per 100 g of diet(normal diet) or tocopherol-free rations which consisted of components similar to those of the normal diet, except for the absence of tocopherol. After feeding for seven months, rats were dissected under ether anesthesia. Then organ-physiological saline homogenates were prepared with a glass homogenizer under ice-cold conditions, and they were immediately submitted to ultraweak chemiluminescent assay by our super-high sensitivity photon counting system.

To further elucidate the chemical species involved in this ultraweak photon emission, the effects of addition of d-α-tocopherol (200 μM) DABCO (1,4-diazabicyclo [2,2,2] octane) (100 μM), and BHT (butyl hydroxytoluene) (100 μM) were investigated, besides the effect of D_2O substitution. Results are shown in figure 7. It was observed clearly (curve A) that the chemiluminescence

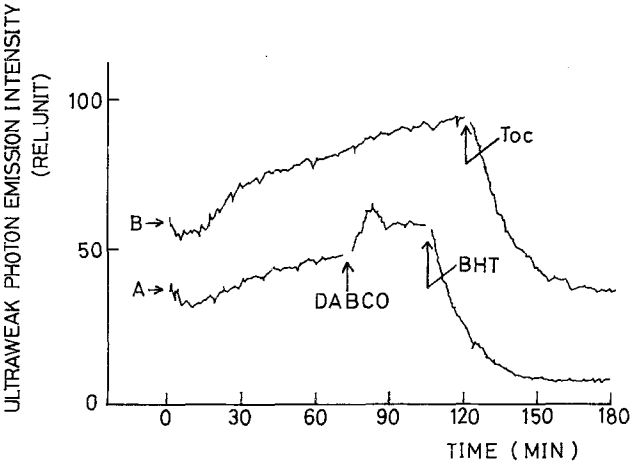

Figure 7. Measured results of ultraweak photon emission intensity from the liver homogenate prepared from tocopherol-deficient rats, and their temporal changes by the addition of d-α-tocopherol (Toc), DABCO and BHT. *A* Tocopherol-deficient liver, 500 mg, homogenized with 4.5 ml of physiological saline, and *B* tocopherol-deficient liver, 500 mg, homogenized with 4.5 ml of 0.9% NaCl–D_2O.

intensity is enhanced by DABCO, which is known as a stimulant for the generation of singlet oxygen molecules ($^1O_2$), while it is effectively quenched by the presence of BHT, acting as a free radical scavenger. Moreover, when the tocopherol-deficient liver was homogenized with D_2O-substituted physiological saline, the photon emission intensity was markedly enhanced (curve B), and this very weak chemiluminescence was diminished appreciably by the addition of tocopherol. It is to be mentioned here that D_2O possesses an elongating effect on the lifetime of $^1O_2$ molecules, and tocopherol is known to quench $^1O_2$ molecules and free radicals. Hence the results presented in figure 7 verify the involvement of excited $^1O_2$ molecules and free radicals in the ultraweak photon emission from the liver homogenate of tocopherol-deficient rats.

A similar study in vitro was also performed employing a model system consisting of a rat liver-physiological saline homogenate and varying amounts of autoxidized linseed oil (AOLO)[9, 15]. We have confirmed experimentally that the ultraweak photon emission from this system is strongly enhanced by the addition of AOLO, which induces the progression of tissue lipid peroxidation, and its intensity is proportional to the amount of AOLO. Figure 8 shows the measured spectral distribution of this extra-weak chemiluminescence, and also the quenching effect resulting from the addition of β-carotene to this system. In this case, the system was prepared with 4 g of rat liver and 20 ml of physiological saline containing 0.02% of sodium cholate, and 1.5 ml of AOLO was added. The AOLO with a POV value of 10^3 meq/kg was obtained by aeration at room temperature. Then the combined system was mixed well by rehomogenation and subjected immediately to spectral analysis.

The measured spectrum presented by the solid curve in figure 8 is distributed through almost the whole visible wavelength region and has three emission maxima at around 520, 575 and 640 nm, with an additional faint emission peak around 480 nm. The five vertical arrows in the upper part correspond to the locations of the emission or absorption spectra of the vibrational bands due to the simultaneous transitions in pairs of excited singlet molecular oxygen ($^1O_2 - {}^1O_2$)[7, 12, 28].

On the other hand, the dotted curve illustrated in figure 8 shows the measured spectral distribution from the mixture after incubation for 30 min in the liver homogenate-AOLO system with the addition of β-carotene, which is known to quench $^1O_2$ molecules. Thus it was demonstrated spectroscopically that in the presence of β-carotene, the original spectrum of this model system is reduced effectively by the disappearance or the quenching of emission bands corresponding primarily to the simultaneous transitions in $^1O_2$ pairs. Moreover, we have observed that the total intensity of the spectrum was quenched by the addition of other $^1O_2$ quenchers, such as tocopherol, hydroquinone and dimethyl furan, and also free radical scavengers [15]. Among them, however, β-carotene provided the most effective quenching in this model system. Because of the existence of an unquenched part of the spectra in these measurements, the involvement of ultraweak photon emission from carbonyls and/or other unknown species was also considered, but not examined in detail.

From these in vitro experiments, it could be conjectured that $^1O_2$ molecules are generated and act as a major photon-emitting species of the ultraweak chemiluminescence in the model system of rat liver-homogenate and AOLO mixture. A possible mechanism for producing $^1O_2$ molecules is likely by the decomposition of peroxy radicals accompanying the self-reaction derived from AOLO in the liver homogenate.

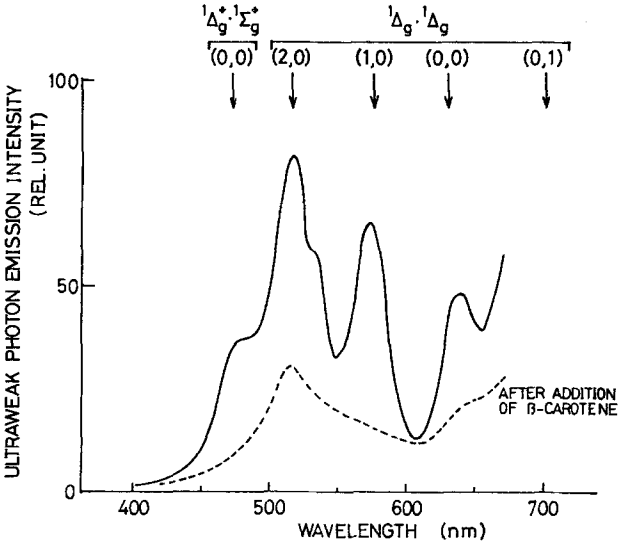

Figure 8. Measured spectral distribution of ultraweak photon emission from the rat liver homogenate on adding autoxidized linseed oil (AOLO) and its change caused by the addition at β-carotene to the sample.

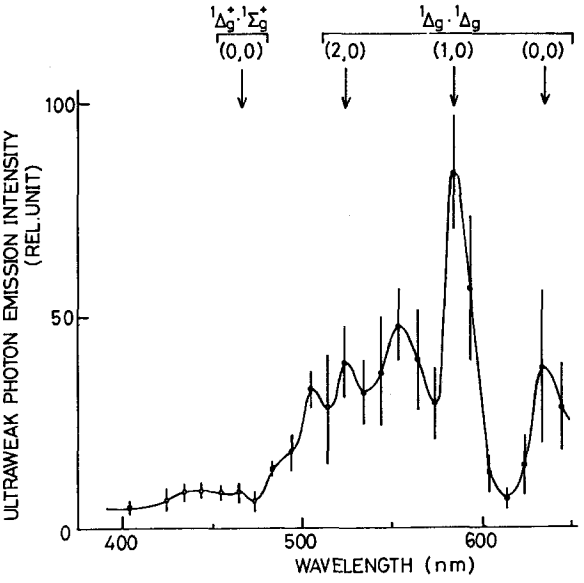

Figure 9. Measured spectral distribution of ultraweak photon emission from the liver homogenate prepared from rats administered intragastrically autoxidized linseed oil (AOLO).

Further investigation was pursued based on these findings, which suggest the generation of $^1O_2$ molecules in lipid peroxidation in vivo, induced by the AOLO administration to rats. In figure 9, the result of spectral analysis is illustrated for a liver homogenate prepared from rats dosed with AOLO[17]. This sample was obtained from male Wistar rats, weighing about 100 g, which were orally treated with AOLO (0.3 ml/rat/day) with a POV value of 10^3 meq/kg for two days. The total intensity of the ultraweak photon emission from this sample was found to have increased about three-fold, compared to the control sample from rats without AOLO feeding. TBA reactive products accumulated at the same time. This enhanced photon emission was quenched effectively by the in vitro addition of free radical scavengers such as BHT, d-α-tocopherol and 2.5-diphenyl furan, whereas superoxide dismutase, catalase and D-mannitol yielded only negligible inhibition of this ultraweak emission.

In figure 9, two emission peaks located around 585 and 635 nm and one broad emission band are shown. These two emission peaks are considered to correspond to vibrational bands associated with the simultaneous transitions in $^1O_2$ pairs as indicated by the vertical arrows. Emission bands at about 480, 520, 580 and 640 nm were reported for the ultraweak chemiluminescence from a reconstituted microsomal system undergoing lipid peroxidation[19], and from the reaction of linoleic acid and Ce^{2+}, which yields lipid peroxy radicals[27]. Hence it is inferred that these two emission lines are due to $^1O_2$ pair molecules as a product of tissue lipid peroxidation. The broad emission band between 500 and 560 nm in figure 9 seems to appear characteristically in heterogeneous biological systems undergoing lipid peroxidation. For this broad band, $^1O_2$ molecules would contribute in some degree to the emission around 520 nm and the emission due to excited carbonyl compounds may also be involved. Excited state carbonyls are thought to be generated in the chain-termination process of hydrocarbon oxidation and to emit ultraweak chemiluminescence with a maximum in the 520–540 nm region[24]. Excited protein molecules, possibly produced as a result of free radical reactions, may also contribute to ultraweak photon emission[13]. From these results, ultraweak tissue chemiluminescence presented in this section could be explained as being due to photoemissive reactions involving free radicals and $^1O_2$ molecules, in which the latter are basically generated by the self-reaction of peroxy radicals derived from lipid peroxides, as a response to oxygenation.

Conclusion

In this paper, the modern technology and systems for the detection and spectral analysis incorporated in the superhigh sensitivity photon counting technique are described for the measurement of the ultraweak photon emission involved in the extremely weak bioluminescence and chemiluminescence from living cells and tissues closely related to biochemical and biophysical activities and processes. Various studies reviewed in this paper, in which these systems have been used to measure the ultraweak photon emission from rat blood samples and organ homogenates, could provide support for the usefulness of this technology as a monitoring method for chain reactions of tissue lipid peroxidation accompanied by the generation of $^1O_2$ molecules and free radicals in living tissues and cells.

Although we have achieved in practice the excellent high sensitivity of one photoelectron per second or better in the photon counting detection procedure, further improvements will be required in order to explore more widely the essential features and roles of the ultraweak cell and tissue photon emission. Our biophoton research project is actively pursuing research into and development of the technology, including both hardware and software, with this aim in view. We hope to collaborate with active workers in this field all over the world, and also to report further results and discussions in future publications.

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Luminescence research and its relation to ultraweak cell radiation

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Summary. The fundamental laws of photochemistry and the essential results of experimental research on ultraweak cell radiation are presented. By comparing all the facts it can be concluded that the phenomena discussed may arise from a variety of possible reactions and sources. Recombination reactions of certain radicals actually do release sufficient energy to generate UV-photons of the intensity under consideration. On the other hand, stimulated emission cannot be excluded in view of the distinct deviation of the radiation field from thermal equilibrium. There exist, however, various other candidates, such as direct emitters like flavins, indoles, porphyrins, carbonyl derivatives and aromatic compounds, and molecular oxygen and its various species, as well as collective molecular interactions, e.g. dimole or exciplex transitions, triplet–triplet annihilation, collective hydrolysis, electric field effects in membranes, etc.

Careful biochemical and biophysical experiments are still necessary to find answers to all the questions that remain; not only individual problems have to be solved, but it is important to keep in mind the interrelationships between certain reactions.

Key words. Bioluminescence and weak photon emission from biological systems; excited states; photochemistry; radical reactions; energy transfer; collective interactions; temperature hysteresis.