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# Highly Sensitive Detection and Spectral Analysis of Ultraweak Photon Emission from Living Samples of Human Origin for the Measurement of Biomedical Information.†

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Ultraweak photon emission originating from living systems and organisms is generally calledbiophoton emission and has known to occur naturally in conjunction with various vital processes of life. The aim of our study is to develop new techniques for biomedical measurements and analyses based on highly sensitive detection and characterization of biophoton emission from living samples of human origin. In this paper, we report the fundamental techniques and instrumentation developed newly for the measurement of ultraweak photon emission intensityand its spectral distribution together with basic characteristics of ultraweak photon emission from blood plasma, urine, sputum and breath samples taken from various subjects.

**Key Words:** biophoton, ultraweak photon emission, blood plasma, urine, sputum, breath

## 1. Introduction

It has been recently accepted that all living organisms emit ultraweak light during ordinary metabolic processes. The phenomenon, referred to as biophoton emission, originates in the chemical excitation of molecules undergoing oxidative metabolism. The intensity of biophoton emission is generally less than approximately $10^{-15} W/cm^2$ and is observed in the visible wavelength range. However, it is distinct from thermal radiation arising from body temperature, which is approximately 1/100 or 1/1000 less than biophoton intensity in the visible wavelength. Biophoton phenomena, observed in various biological materials under versatile conditions have been surveyed from cellular or subcellular levels up to individual organism level, following the development of the highly sensitive photon detection technique. These studies suggest the potential usefulness of the biophoton, which reflects the pathophysiological states of organisms in each level of biological hierarchy, to extract biomedical information relevant for diagnosis [1)~6)]. In this paper, we report the exploration of a novel technique for biomedical measurement based on biophoton analysis, with characterization of various samples in human.

## 2. Mechanism and characteristics of biophoton emission

A biophoton is a spontaneous photon emission, without any external photo-excitation, through chemical excitation of the internal biochemical processes underlying cellular metabolism [1)~4)]. Usually, the mechanism of the biochemical reaction process is related to the oxidative metabolism, which accompanies the generation of reactive oxygen species (ROS). Many cases of biophoton phenomena have been discussed with respect to radical reactions through ROS generation and ROS initiated cellular dysfunction. For instance, in the process of lipid peroxidation, excited species such as carbonyls and singlet-oxygen are generated during a radical chain reaction triggered by ROS (Russel mechanism) [4)]. These excited species and/or other fluorescent molecules excited through energy transfer, are thought to lead to biophoton emission [4)~6)]. In general, biophoton emission is distinguished from general bioluminescence phenomena such as that observed in firefly. The difference lies in the luminescent mechanism with photon yielding efficiency resulting in an intensity difference of over $10^3$. Bioluminescence generally originates in enzymatic reactions such as the luciferin-luciferase system, whereas, biophoton emission is caused by various mechanisms and species accompanying inherent oxidative metabolism. Recently oxidative stress through ROS generation has been generally recognized as being related to various diseases, which are derived from oxidative modification of cellular constituents such as lipid, protein, nucleic acid, and enzymes. Hence, biophoton emission

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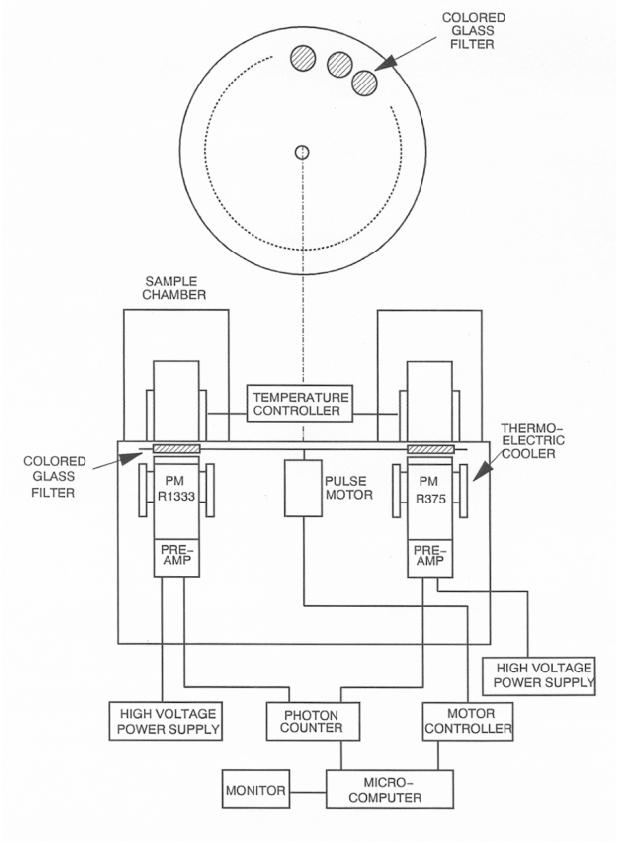


**Fig. 1** Block diagram of the filter-differential type biophoton spectral analyzer system.

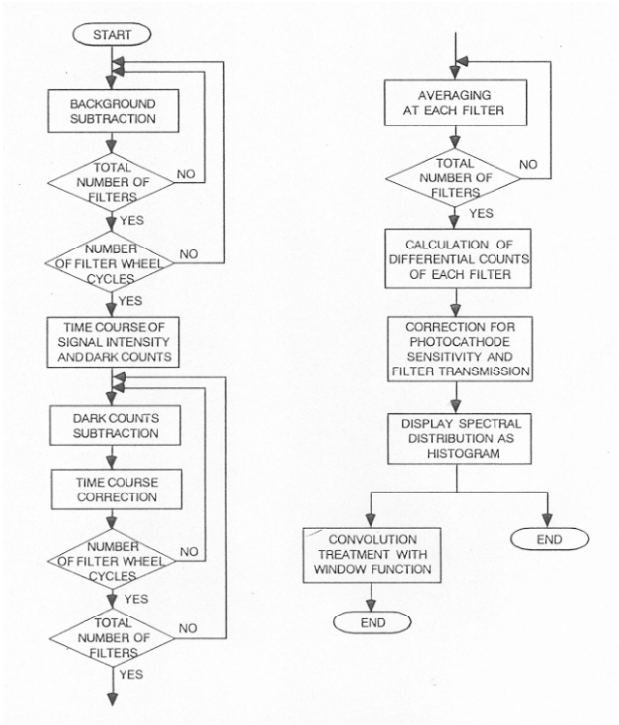


**Fig. 2** Flow chart for extracting the spectral information in the filter-differential type biophoton spectral analyzer system.

might indicate pathological states. For example, it has been reported that UV irradiation on skin, administration of lipid peroxide or carcinogens, and other conditions of oxidative stress, induce an increase in biophoton intensity [1)~4)]. Aging is also known to affect the enhancement of biophoton emission. It implies that biophoton emission is an indicator of imbalance between oxidation and antioxidative protection, which is illustrated as the excess production of ROS and/or decline in the activity of antioxidation. Detection of biophoton emission can provide real-time characteristics of various biological samples from individual organisms to the cellular and material levels as can metabolic products.

## 3. Spectroscopy methods for the determination of ultraweak biophoton emission

We have developed a prototype spectral analyzing system (the filter-differential type biophoton spectral analyzer system) for ultraweak light emission over a wide wavelength range. Photomultiplier tubes (PMT) selected for highly sensitive detection under the operation of single photon counting are used. The system is designed for filter-spectroscopy, which is suited to the properties of biophoton emission with a broad spectrum and little need for high resolution of the wavelength [7), 8)]. Colored glass sharp cut-off filters are used. In our studies, a set of 37 filters, with different cut-offs, from 250 to 850nm was used. Spectral distribution of the sample was calculated as the difference between transmitted intensities of two subsequent filters [9)~11)]. The block diagram of the system is indicated in **Fig. 1**, and a flowchart of the computation procedure to derive the spectral distribution, is shown in **Fig. 2**. Schematic illustrations of the process are also displayed in **Fig. 3**. The system has two detectors with different spectral responses for over a wide range of measurements. A PMT R1333 (Hamamatsu Photonics KK) is used for the range of 300-900nm and an R375 (Hamamatsu) is for 160-650nm. Filters are mounted on a rotating disk inserted between the sample chamber and the PMTs. One cycle of the rotating disk provides a set of transmitted intensity data through all filters including the dark count of the PMT and total intensity over the wavelength range, which is measured without any filters. An illustrated figure of raw data obtained with repeated disk cycles is shown in Fig. 3(a) with temporal changes of the total intensity and dark counts acquired in each rotation (Fig. 3(b)). After subtraction of the background

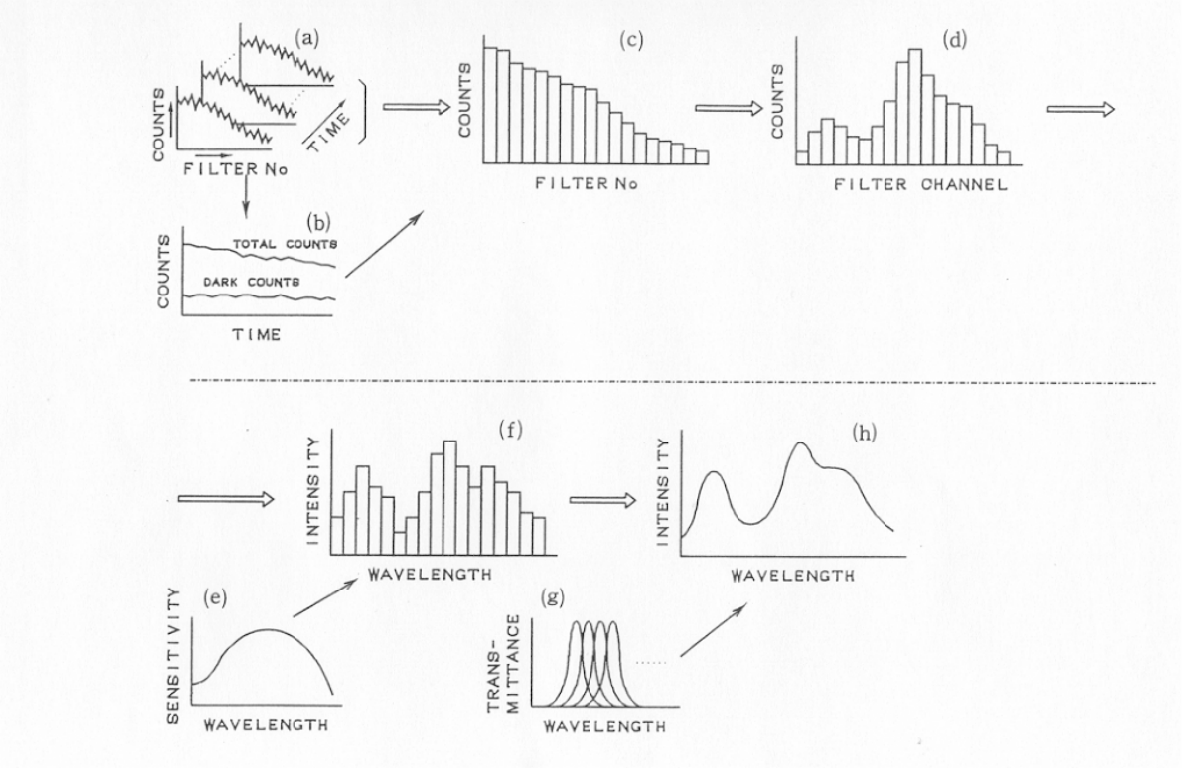


**Fig. 3** Schematic illustrations of the data processing in the filter-differential type biophoton spectral analyzer system.

emission of the filters, correction of temporal changes of the total intensity and dark counts are carried out. Integrations of the intensity obtained for each filter are then calculated as shown in Fig. 3(c). Figure 3(d) is the result after calculating the difference between subsequent data. Correction of spectral response (Fig. 3(e)) of the PMT is also applied, and then the spectral distribution is expressed as a histogram, as shown in Fig. 3(f), with the wavelength resolved by the cut-off properties of each filter. For visualization, if necessary, the spectral distribution can be displayed as a curve as shown in Fig. 3(h) through convolution treatment using a window-function for the filters (Fig. 3(g)), which is obtained by subtraction of the transmittance curves from two subsequent filters. Consequently, the wavelength resolution of the system is approximately 20-30 nm through the range 300-700 nm and 30-50 nm in the other wavelengths region.

## 4. Highly sensitive detection and spectral analysis of ultraweak photon emission from human originated samples.

We examined the potential application of biophoton analysis in physiological diagnosis. Samples of blood, urine, sputum, and breath derived from human volunteers were investigated. **Table 1** shows a comparison of biophoton emission from these samples [12]. Here we describe the results obtained from the viewpoint of methodological examination for applying to laboratory tests.

### 4.1 Blood plasma

It is known that detection of ultraweak photon emission of blood or blood plasma is dependent on the pathological states of the human body [1)~4)]. For example, in the case of smoker's blood, photon emission intensity of the plasma shows a high intensity value, suggesting a contribution from free radicals in the smoke [13]. We have studied the photon emission characteristics of blood plasma obtained from hemodialysis (HD) patients, who were known to be under oxidative stress [14]. As shown in **Fig. 4**, which is a comparison of intensities between HD patients and normal volunteers, the photon emission intensity of plasma from HD patients is approximately 1.5 fold higher than normal [10), 12), 15)]. The high intensity implies a reflection of the oxidative stress that occurs in HD patients. A spectral comparison is shown in **Fig. 5**, indicating the different distribution pattern in the dominant wavelength region at 500-700 nm, which appeared in HD patients. **Figure 6** shows a spectral comparison of blood plasma obtained after the treatment to induce autooxidation under an oxygen atmosphere, in comparison with that under argon.

**Table 1** Comparison of ultraweak photon emission intensities and spectral characteristics of typical biomedical samples of normal (healthy) human origin

| SAMPLE | EMISSION INTENSITY (counts/10 s)+ | EMISSION INTENSITY (x $10^3$photon/s)++ | EMISSION INTENSITY (x $10^{-16}$W)++ | SPECTRAL RANGE (PEAK WAVELENGTH) (mm) | MEASUREMENT CONDITION |
|---|---|---|---|---|---|
| BLOOD PLASMA | 800～1500 | 2.2～4.1 | 7.0～13 | 500～700 (530*, 630*, 670) | Non-smoker, 2.5 m*l* |
| URINE | 300～1500 | 0.9～4.4 | 2.8～14 | 450～650 (490, 530*, 630) | 2.5 m*l* |
| SPUTUM | 2000～10000 | 5.8～28.8 | 19～90 | 450～700 (530*, 610, 670) | 1 g |
| SALIVA | 400～800 | 2.4～4.9 | 8.6～17 | 450～650 (530*, 610) | 2.5 m*l* |
| BREATH | 400～600 | — | — | — | 1.8 *l*/min. |
| HUMAN BODY SURFACE | 500～800 | — | — | 500～700 (550, 630～670*) | Finger tip, 2 cm diameter |

* At peak wavelength.
\+ Obtained from actual measurements.
++Obtained from emission spectra after correction for light collection efficiency.

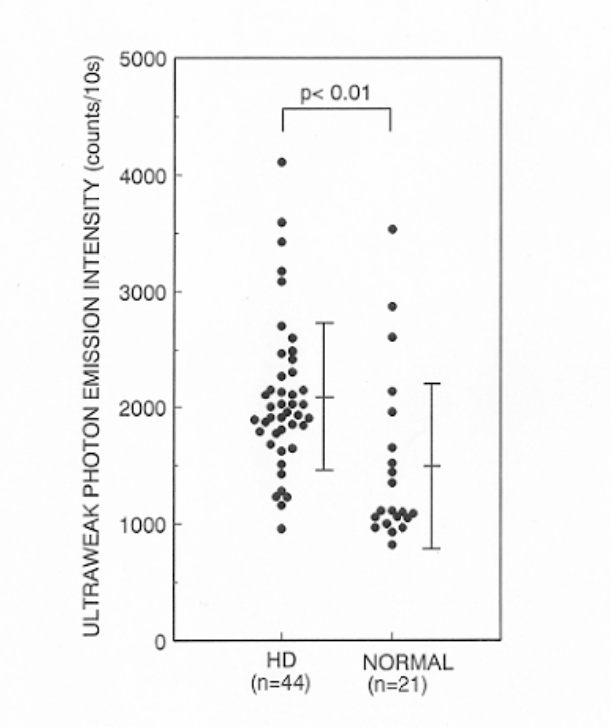


**Fig. 4** Comparison of ultraweak photon emission intensity of blood plasma samples taken from hemodialysis (HD) patients and normal subjects.

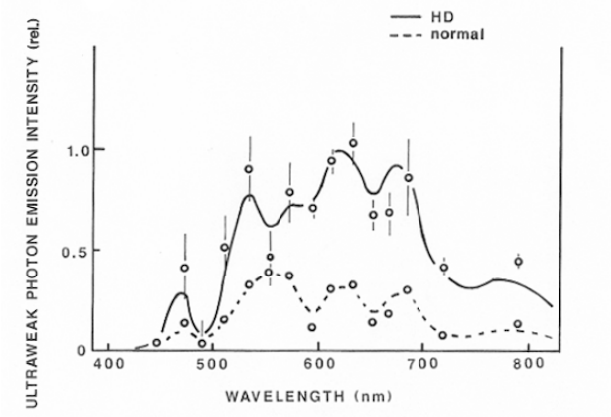


**Fig. 5** 5 Ultraweak photon emission spectra of blood plasma samples taken from HD patient and normal subject.

**Figure 7** is the spectral comparison of plasma under the treatment of high alkalinity (pH 11) and of bililubin IX (Sigma Co.) dissolved in an alkaline solution at pH 11. A specific emission peak under the oxygen atmosphere or alkaline treatment appeared at 670 nm, corresponding to the wavelength observed in the emission peak of bililubin IX. It suggests a contribution from bililubin for the excited species emitting at 670 nm. On the other hand, administration of bililubin IX to neutral plasma also induces the specific increase in intensity at 670 nm, supporting the contribution of bililubin. Although emission species that contribute to the other wavelength region have not been well clarified, the contribution of lipoprotein and albumin through the oxidative excitation is suspected. As indicated in these results, plasma photon emission data, especially that of the wavelength, might be useful for the extraction of physiological and pathological information.

### 4. 2 Urine

Urine, which contains certain final metabolic products, is convenient to use in a laboratory test. It is known that some inflammatory diseases cause an increase in the photon emission intensity of urine[3),4)]. The correlation between the photon emission intensity and concentration

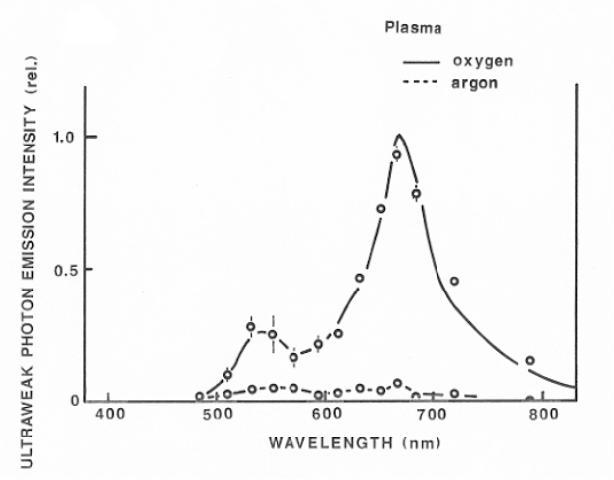


**Fig. 6** Ultraweak photon emission spectra of blood plasma sample taken from normal subject under oxygen and argon gas atmospheres.

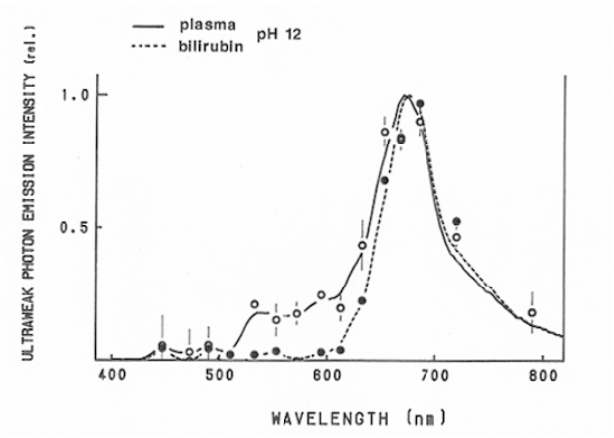


**Fig. 7** Ultraweak photon emission spectra of blood plasma sample and bilirubin under an alkaline condition (pH 12).

of urobilinogen or the CRP value has been reported. The influence of smoking has also been observed, similar to that in blood plasma[3),4)]. However, since photon emission intensity depends on the water content and pH[16)], preparation of normalized samples is necessary, prior to our examination of the variation in spectral distribution for characterization. **Figure 8** shows a typical example of a photon emission spectrum obtained from a normal volunteer. Although the spectral pattern resembles that of blood plasma,an enhancement at 670 nm under an oxygen atmosphere, which is obtained in plasma, was not observed. We studied the temporal changes of the spectral pattern in a series of urine samples obtained over one day from a normal volunteer. However, variation among their spectral patterns, except for intensity changes, was not observed. This reproducibility indicates the validity

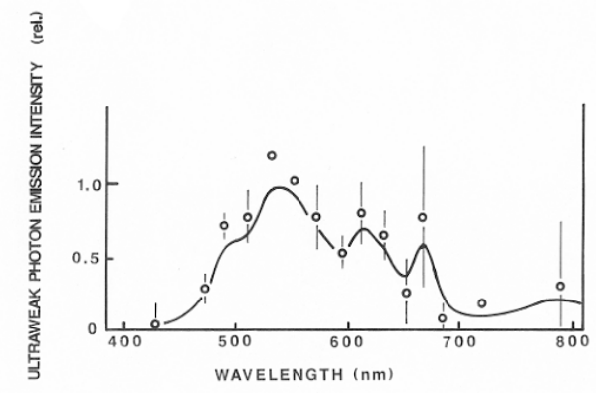


**Fig. 8** Ultraweak photon emission spectrum of a urine sample taken from normal subject.

of the spectrum information applied as a screening test, when the relationship between spectral changes and some diseases are revealed.

### 4.3 Sputum

Leukocytes and macrophages are known to emit ultraweak light during phagocytosis, corresponding to ROS generation, which is a function of cellular immunity. From spectral analysis studies exploring this biochemical mechanism, the contribution of amino acid oxidation processes initiated by superoxide generation has been revealed[17)]. We have examined the application of these phenomena for use in a clinical test using sputum. Extracted sputum, which contains cellular components, is expected to reflect the physiological status of the lungs and bronchial tubes, e.g., inflammation, as differences in photon emission intensity. **Figure 9** shows a typical spectra of ultraweak photon emission of sputum compared with saliva, indicating the distinct patterns of both. We found that photon emission increased after a surgical operation from clinical data collected from patients over one week[10),18)]. This might be linked to an inflammation. Although the detailed mechanism of photon emission and the contribution of cellular components in sputum is not clear, this method has potential usage in a clinical test of primary monitoring for screening.

### 4.4 Breath

Breath contains variable minor components produced in the metabolism. Ultraweak photon emission from exhaled gases was reported as being derived from excited molecules in these minor components[9),18),19)]. ROS were considered to be the initial species contributing to biophoton emission, which were generated under oxidative metabolism mainly in energy yielding processes. The aim of our study on breath photon emission was to develop a quantitative analysis of oxidative stress on a whole body.

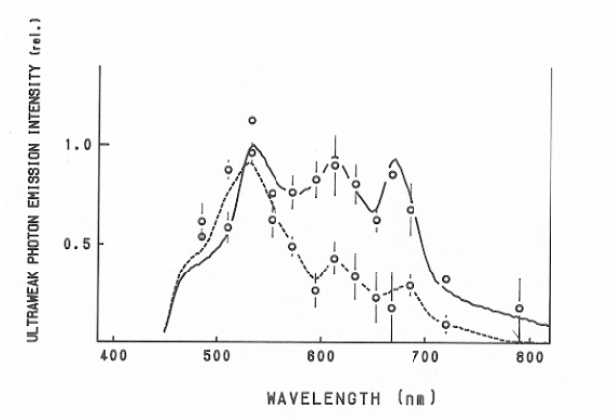


**Fig. 9** Comparison of ultraweak photon emission spectra of sputum and saliva taken from normal subject.

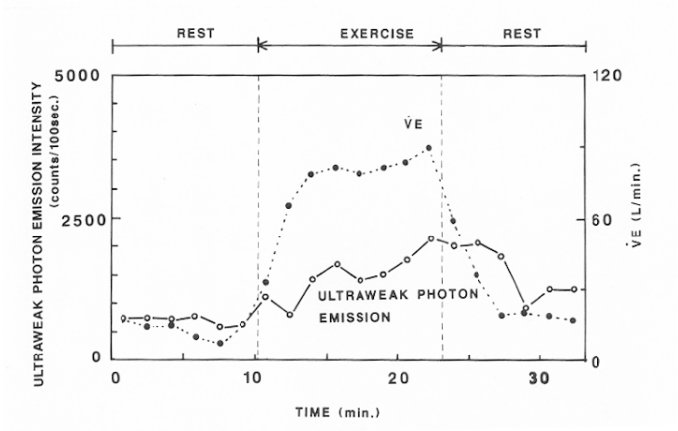


**Fig. 10** Temporal changes of ultraweak photon emission intensity of expiration from normal subject and minute ventilation ($\dot{V}E$) during exercise with a bicycle ergometer between rests.

Here we describe the results of experiments obtained under oxidative stress induced by excessive exercise. Intense exercise is known to cause oxidative stress, which is indicated by an increase of lipid peroxidation in blood plasma or the generation of pentane or ethane in the breath, as the final products of the decomposition of lipid peroxides. The mechanism is considered to be through ROS generation in the electron transfer chain, explained as electron leakage induced by the anoxia or temporal ischemia in cardiac muscle[20]. Typical data from an experiment obtained during excessive exercising of a normal volunteer is shown in **Fig. 10**, which indicates the intensity variation of photon emission compared with ventilation volume per minute. Exercise was continued over 15 minutes under the conditions needed to exceed the anaerobic threshold. As indicated in the figure, the gradual increase of photon emission intensity compared with an abrupt increase of ventilation was observed. The relationship between the photon emission intensity and ventilation rate resulted in the hysteresis pattern, which represented the delay of the photon emission response after the increase in ventilation rate. It is considered that the characteristics of the delay imply the physiological response to the excess exercise. The relationship between the response of photon emission and propagation of lipid peroxidation are proposed, demonstrating the potential usefulness of photon detection for real time monitoring of physiological states under oxidative stress. It might be applicable for general diagnosis regarding oxidative stress, not only for sports medicine. We also have been investigating a method of effective capturing photons in breath through collection by cold trapping.

## 5. Conclusion

We describe the studies of novel techniques based on the detection and analysis of spontaneous photon emission in various samples originating from humans, for non-invasive monitoring of the body. Due to the complexity of the emission species of living samples, the details of emission mechanisms are not yet clarified in detail. However, photon detection is an attractive method for the convenient monitoring of oxidative stress as a primary health screening method, in consideration of the social requirement for welfare, especially for an aging society. The biophoton, which indicates the vital activity of organisms, will provide information from raw data without external perturbation. For diagnostic applications, it is also necessary to develop new techniques on the hardware side including detector and analysis systems, such as the dynamics of photon emission spectrum with spatial information. The merits of this method using the biophoton phenomena are the simplicity of sample preparation, and excellent sensitivity with reproducibility that are inherent with the light detection technique, resulting in it being applicable for automated screening tests or real-time monitoring. It is expected to be valid for novel diagnostic and biomedical measurements with further progress in this technique accompanied by the creation biophoton and disease correlation databases.

### References

1) H. Inaba: Biophoton, BME (Jpn. J. Med. Electr. Bio. Eng.), 1-8, 628/635 (1987) (in Japanese)
2) H. Inaba: Biomedical application of the measurement of ultraweak photon emission, Jpn. J. Optics (Kogaku), 12-3, 166/179 (1983) (in Japanese)

3) H. Inaba: Measurement of ultraweak biophoton emission, Byotai-seiri, 9-2, 101/110 (1990) (in Japanese)
4) A. Miyazawa and H. Inaba: Ultraweak photon emission, Bioluminescence and chemiluminescence -Basics and Experiments- (K. Imai ed.), p.126. Hirokawa shoten (1989) (in Japanese)
5) H. Watanabe and H. Inaba: Biochemistry of biophoton, -metabolism, mechanism, and evolution- (1), O plus E, 142, 112/123 (1991) (in Japanese)
6) H. Watanabe and H. Inaba: Biochemistry of biophoton, -metabolism, mechanism, and evolution- (2), O plus E, 143, 139/1153 (1991) (in Japanese)
7) Y. Shimizu, Y. Tsuji and H. Inaba: Measurement of ultraweak photon information -theory and application for the spectral analysis of the ultraweak photon emission, Jpn. J. Optics (Kogaku), 4-3, 105/123 (1975) (in Japanese)
8) H. Inaba, Y. Shimizu, Y. Tsuji and A. Yamagishi: Photon counting spectral analyzing system of extra weak chemi- and bioluminescence for biomedical applications, Photochem. Photobiol., 30, 169/175 (1979)
9) M. Kobayashi and H. Inaba: Method for ultraweak photon detection and application for the measurement of biomedical information, Respiration Res. (Kokyu), 9-5, 543/538 (1990) (in Japanese)
10) M. Kobayashi and H.Inaba: Biomedical measurement based on the detection and analysis of ultraweak biophoton emission, Opt. Elecro-opt. Eng. Contact (Hikari-gijyutu contact), 28-12, 670/700 (1990) (in Japanese)
11) M. Kobayashi: Detection of biophoton emission from living samples and measurement of biomedical information, Research report on Inaba Biophoton Project, ERATO, JRDC, p.21, Res. and Develop. Corp. Japan (1991) (in Japanese)
12) M. Kobayashi and H. Inaba: Biomedical measurement by using ultraweak biophoton emission, J. Med. Tech. (Rinsyo-kensa), 35-6, 610/615 (1991) (in Japanese)
13) B. Yoda, Y. Goto, K. Sato, A. Saeki and H. Inaba: Ultraweak chemiluminescence of smoker s̀ blood, Arch. Environ. Health, 40, 148/150 (1985)
14) M. Kuroda, S. Asaka, Y. Tofuku and R. Takeda: Serum antioxidant activity in uremic patients, Nephron, 41, 293/298 (1985)
15) S. Agatsuma, H. Sekino. M. Kobayashi and H. Inaba: Measurement of ultraweak photon emission of blood plasma obtained from hemodialysis patients, Renal Failure Res. (Jinfuzen), 2-1, 81/88 (1990) (in Japanese)
16) M. Kobayashi, S. Agatsuma, H. Sekino and H. Inaba: Measurement of ultraweak photon emission of living samples, Renal Failure Res. (Jinfuzen), 2-1, 73/80 (1990) (in Japanese)
17) M. Nakano: Luminescence of while cell during phagocytosis, Bioluminescence and chemiluminescence -Basics and Experiments- (K. Imai eds.), p.50. Hirokawa shoten (1989) (in Japanese)
18) M. Kobayashi, K. Asai and H. Inaba: Highly sensitive detection of ultraweak photon emission from human breath and sputum, J. Jpn. Soc. Laser Med., 9-3, 527/530 (1988) (in Japanese)
19) M. D. Williams and B. Chance: Spontaneous chemiluminescence of human breath, J. Biol. Chem., 258, 3628/3631 (1983)
20) M. Kobayashi, K. Asai, W. Hida, H. Inoue, T. Takishima and H. Inaba: Measurement and characterization of ultraweak photon emission of human breath during exercise, J. Jpn. Soc. Laser Med., 10-3, 509/512 (1988) (in Japanese)

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