spectrum, which ranged between 450-700 nm included peaks at 490 nm and 530 nm. The spectral pattern was similar to that of the latter stages of oxidation of linoleic acid, except in the emission region at around 450-520 nm.

A comparison of these results with those of the brain slices suggests that arachidonic acid and linoleic acid or their derivatives may be candidates for the excited species involved in the biophoton emission detected from rat brains. The pathway for excitation may include radical reactions through lipid peroxidation with ROS production. However, the peak obtained from the brain slices at 610 nm does not correspond to any of the oxidized fatty acids, indicating the existence of other sources of the emission. We can speculate that these might be oxidative derivatives from proteins or other molecules.

3.4 HUMAN BODY

In order to survey the application of biophotons to the measurement of human subjects, we tried to evaluate potential methods for characterizing the biophoton image of a human body. We also investigated ultraweak photon emission of samples originated in human subjects; blood plasma, urine, and breath were examined [9].

3.4.1 Biophoton imaging of a human body surface [22]

The biophoton emissions detected from a palm, a face and from the upper part of the waist were examined using a two-dimensional photon counting imaging system and a highly sensitive CCD imaging system. Biophoton images that were determined after 30 minutes observation are displayed in Fig. 11 with the image of the subjects under weak light illumination. These images were taken after dark-adaptation for 10 minutes to minimize the effect of delayed fluorescence. The images display the characteristic distribution of the intensity of biophoton emission on the surface of the body. As shown by the image of the upper part of the waist, the photon

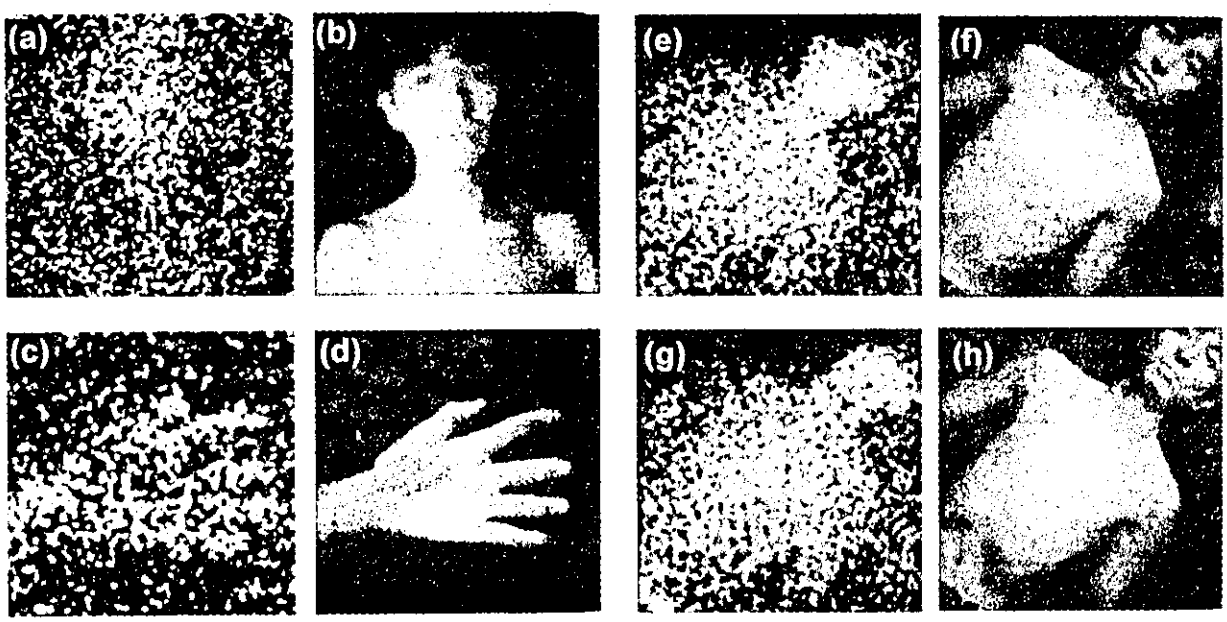

Fig. 11. Biophoton images of a human subject. (a) A biophoton image of a face taken as shown in the photograph (b). (c) A biophoton image of a palm taken as shown in the photograph (d). (e), (g) Biophoton images of the upper part of the waist taken as shown in photographs of (f) and (h), respectively. (a) and (c) were measured with using the two-dimensional photon counting imaging apparatus, and (e) and (g) were measured with the CCD imaging system. All of biophoton images were taken with observation time of 30 minutes.

emission intensity around the face is higher than it is around the body. This may represent a difference in the condition of the skin due to exposure to environmental light. In the case of the face, the intensity in the central area around the nose is observed to be higher. This could be caused by secretions on the skin surface or more speculatively the influence of porphyrin compounds secreted by indigenous bacteria on the skin. In order to estimate the contribution to the photon emission intensity of the autooxidation of secreted sweat or sebum on skin surface, the dependence of the photon-emission intensity on atmospheric oxygen was investigated. We compared the photon emission intensity detected from the palm of a human hand under aerobic and anaerobic conditions by filling the measurement chamber with either pure oxygen or pure nitrogen. The result, which shows the remarkable dependence of the photon-emission intensity on the concentration of oxygen, suggests the contribution of lipid peroxidation occurring on the skin surface, probably including the peroxidation of secreted fluids. The photon-emission intensity from a palm under anoxia was depressed by approximately 25% compared with the value in air and by 50% under pure oxygen. This implies that the photons detected on the body surface contain an atmospheric-oxygen-independent component, originating from sub-epidermal or dermal cells, or perhaps from other cells below the basal layer. The determination of this component may lead to information for evaluating the susceptibility to oxidative stress of dermal cells, which may in turn lead to diagnostic applications for skin medicine. Biophoton imaging is now under investigation to explore applications under various environmental conditions, including exposure to ultraviolet (UV) irradiation, chemical agents and other physical stimuli. Many other studies aimed at the medical application of biophotons from the skin surface have been reported from a variety of viewpoints, including delayed luminescence after UV light exposure [24, 25].

3.4.2 Ultraweak photon emission spectra of blood plasma

We have examined the potential application of ultraweak photon emission to physiological or pathological diagnosis with using human derived samples; blood, urine, sputum, and breath. Table 1 shows a general comparison of photon emission properties of these samples. Here I describe the results obtained for methodological examination aiming to applying for laboratory tests. The measurements of liquid samples, for plasma and urine, were carried out with using a quartz chamber of 2.5 ml volume under 37°C; as to breath sample, a metal cylinder with volume of 500 ml attached on a PMT was used.

It is known that detection of ultraweak photon emission of blood or blood plasma is dependent on the pathological states of the human body. 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 [26]. We have studied the photon emission characteristics of blood plasma obtained from hemodialysis (HD) patients, who were known to be under oxidative stress. As shown in Fig. 12 (a), 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. The high intensity implies a reflection of the oxidative stress that occurs in HD patients. A spectral comparison is shown in Fig. 12 (b), indicating the different distribution pattern in the dominant wavelength region at 500-700 nm, which appeared in HD patients. A spectral pattern of blood plasma obtained after the treatment to induce autooxidation under an oxygen atmosphere or under the treatment of high alkalinity (pH 12), enhanced the emission peak at 670 nm. A solid line in Fig. 13 shows the spectrum under alkaline condition. And this specific peak corresponds to the wavelength of emission peak observed in the chemiluminescence of bililubin IX under oxidation with alkaline treatment (a dash line in Fig. 13), representing a contribution of bililubin for the excited species emitting at 670 nm on the plasma spectrum. Furthermore administration of bililubin IX to neutral plasma also induced the specific increase in intensity at 670 nm with dose dependency, suggesting the participation of bililubin as an indicator of status of

Table 1. Comparison of ultraweak photon emission intensities and spectral characteristics of typical biomedical samples originated form normal (healthy) human subjects. Intensity in the unit of counts/10s was obtained from actual measurements and the unit in watt is calculated based on the spectrum with correction of detection efficiency.

Sample	Photon emission intensity			Spectral range (Peak wavelength) (nm)	Measurement condition
	(counts/10 s)	($x10^3$ photon/s)	($x10^{-16}$ W)		
Blood plasma	800 ~ 1500	2.2 ~ 4.1	7.0 ~ 13	500 ~ 700 (530*, 630*, 670)	Non-smoker, 2.5m*l*
Urine	300 ~ 1500	0.9 ~ 4.4	2.8 ~ 14	450 ~ 650 (490,530*, 630)	2.5m*l*
Sputum	2000 ~ 10000	5.8 ~ 28.8	19 ~ 90	450 ~ 700 (530*, 610,670)	1g
Saliva	400 ~ 800	2.4 ~ 4.9	8.5 ~ 17	450 ~ 650 (530*, 610)	2.5m*l*
Breath	400 ~ 600	–	–	–	1.8*l*/min
Human body	500 ~ 800	–	–	500 ~ 700 (550,630~670*)	Finger tip, 2cm diameter

* A primary peak in the spectrum

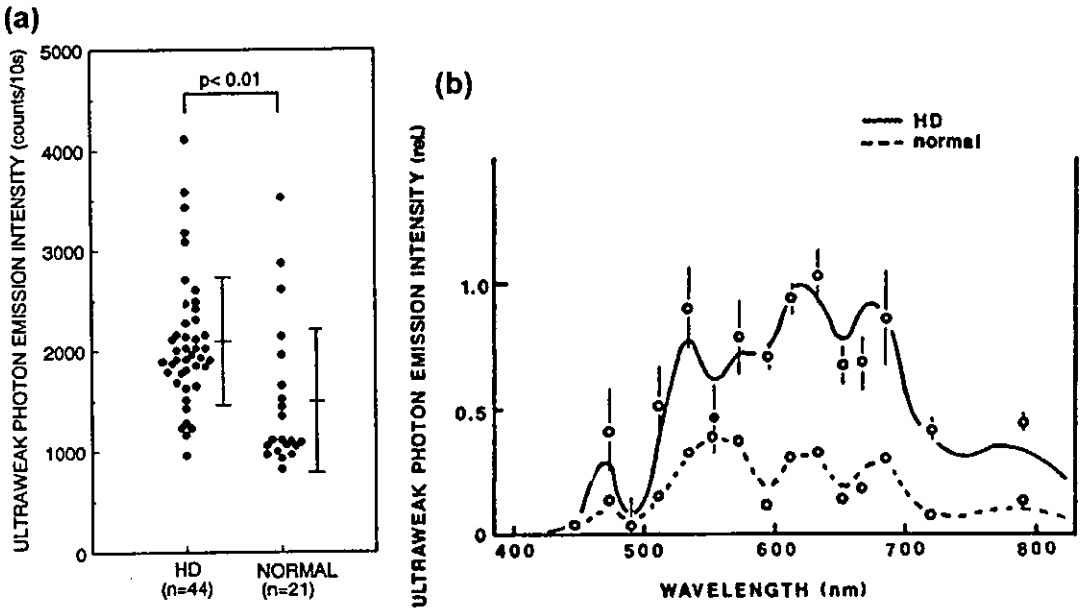

Fig. 12. (a) Comparison of ultraweak photon emission intensity of blood plasma sample taken from hemodialysis (HD) patients and normal subjects. (b) Spectral comparison of ultraweak photon emission of blood plasma taken from HD patients with normal subjects.

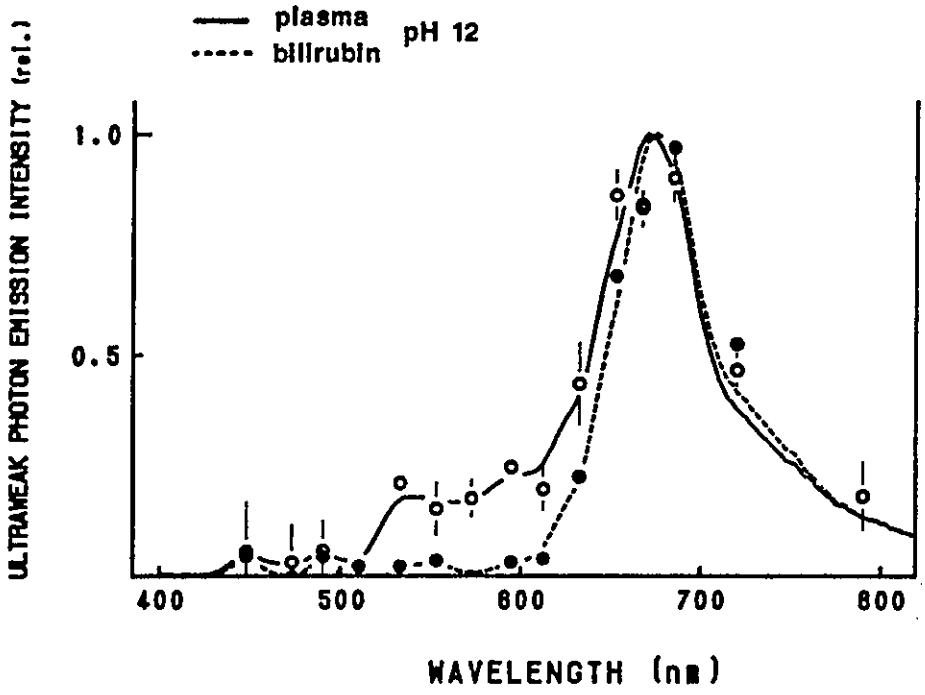

Fig. 13. Ultraweak photon emission spectra of blood plasma taken from normal subject under alkaline condition (pH 12) (solid line) with its comparison to that of chemiluminescence of bilirumin IX under oxidative condition of pH12 (dash line).

oxidation on the plasma. 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. The difference of the spectral pattern between HD patients and normal subjects, as enhancement of the wavelength region at 600-700 nm, may reflect the oxidative stress on which HD patients suffer. These results indicate that plasma photon emission, especially the spectral information, might be useful for the extraction of physiological and pathological information.

3.4.3 Ultraweak photon emission spectra of 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. The correlation between the photon emission intensity and concentration of urobilinogen or the CRP value has been reported. The influence of smoking has also been observed, similar to that in blood plasma. However, since photon emission intensity depends on the water content and pH, preparation of normalized samples is necessary, prior to our examination of the variation in spectral distribution for characterization. The spectral pattern of normal urine sample (data not shown) resembles that of blood plasma; however an enhancement at 670 nm under an oxygen atmosphere, which was 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 of the spectrum information applied as a screening test, when the relationship

between spectral changes and some diseases are revealed.

3.4.4 Ultraweak photon emission of breath under the induced oxidative stress

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 [27]. 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 this study on breath photon emission was to develop a quantitative analysis of oxidative stress on a whole body. Here the results of experiments obtained under oxidative stress induced by excessive exercise are described. 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.

Typical data from an experiment obtained during excessive exercising of a normal volunteer is shown in Fig. 14, 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

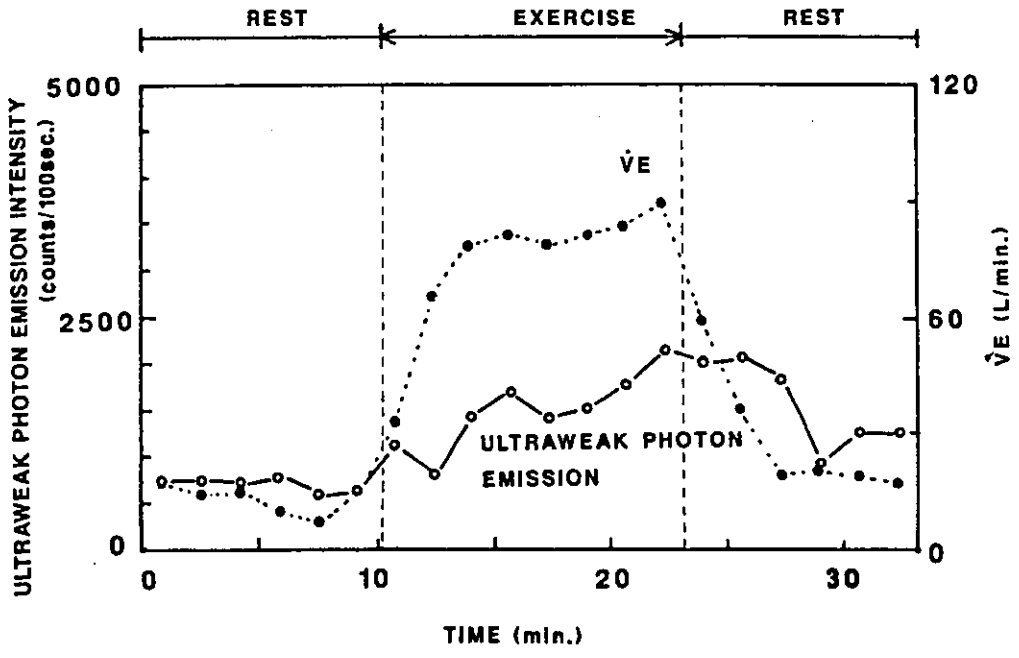

Fig. 14. Temporal changes of ultraweak photon emission intensity of expiration from a normal subject and minute ventilation (V̇E) during excessive exercise with a bicycle ergometer between rests.

photon detection for real time monitoring of physiological states under oxidative stress. It might be applicable for general diagnosis regarding oxidative stress induced with the other causes.

4. BIOPHOTON STATISTICS [4]

Biophoton phenomena are incorporated into the metabolic processes of life, and might accompany their properties originated in intensity fluctuations originating from the interplay among elementary biochemical reactions under physiological conditions. A biological system is understood as a highly integrated organization from a subcellular molecular level up to a macroscopic level, illustrated as the hierarchy structure. It was believed that complex regulation circuitry and interactions over the hierarchy emerge as macroscopic spatial, temporal, or functional structures, expressed as self-organizing or self-regulating. Hence, photon emission properties that are derived from biochemical excitation processes will carry information regarding feedback or Markovian reaction processes that consist of elementary reactions. The analysis of photon statistics or photon correlations is hence expected to extract novel information on biological system.

We studied the methodology for the analysis of photon statistics of ultraweak light spontaneously emitted from chemically excited states within a biological system. Temporal characteristics of photon statistics in biophoton emission from cellular slime mold (*Dictyostelium discoideum*) during the early stage of development have been studied as the model experiment applying biophoton statistics analysis.

In our previous studies, properties of the super-Poisson statistics of photon emission observed in bioluminescent bacteria (*Photobacterium phosphoreum*) during the early stage of synchronous proliferation, in which statistical properties were quantified by the characterization of the Fano factor [11]. This was suggested to be due to photon clustering, which is associated with the genetic expression of enzymes (luciferase and/or aldehyde-synthesis enzymes) in the reaction circuit for the luminescence, and which is interpreted as an abrupt generation of excited intermediators that are derived by the inductive activation of luciferase synthesis in the early stage.

We aim to apply the photon statistics analysis to biophoton phenomena and focussed on biological phase transitions such as multicellular development, which may be characterized by means of intercellular cooperation, signal transduction, and synchronous gene expressions. To investigate the potential characterization of biological cooperation by means of quantum stochastic analysis, we studied the emission properties of microorganisms during development. In the experiments, we used *Dictyostelium discoideum* known as a useful model organism for investigation of cellular differentiation in developmental biology. *D. discoideum* shows the characteristic pattern of a well-defined life cycle including distinct periods for the growth and developmental stage. With an abundant food supply, these organisms live in a colony of amoebae as independent cells that multiply; however, when the food supply is exhausted, their proliferation stops and the single cells collect into multicelluar aggregates with a composition of about 10^5 cells, and each aggregate subsequently undergoes a developmental process to become a slug and finally forms a fruiting body.

Temporal augmentation of ultraweak photon emission during developmental stage had been known and suggested that the production of ROS would be caused by perturbation of mitochondrial oxidative metabolism, and speculating the intracellular stress signal for controlling gene expression under differentiation [28]. We have analyzed a temporal increase of biophoton emission within 1 hour after starvation and subsequent augmentation of photon emission characterizing the behavior of intercellular cooperation related with ROS generation.

Figure 15 shows a typical example of the intensity time course of photon emission after starvation. An emission intensity peak appeared 10-20 minutes after the starvation, and the photon emission intensity decreased to a steady level within 30 minutes. During this period, the morphological changes to the aggregation stream had not yet begun.

We analyzed the temporal variation of photoelectron statistical properties as the observation-time

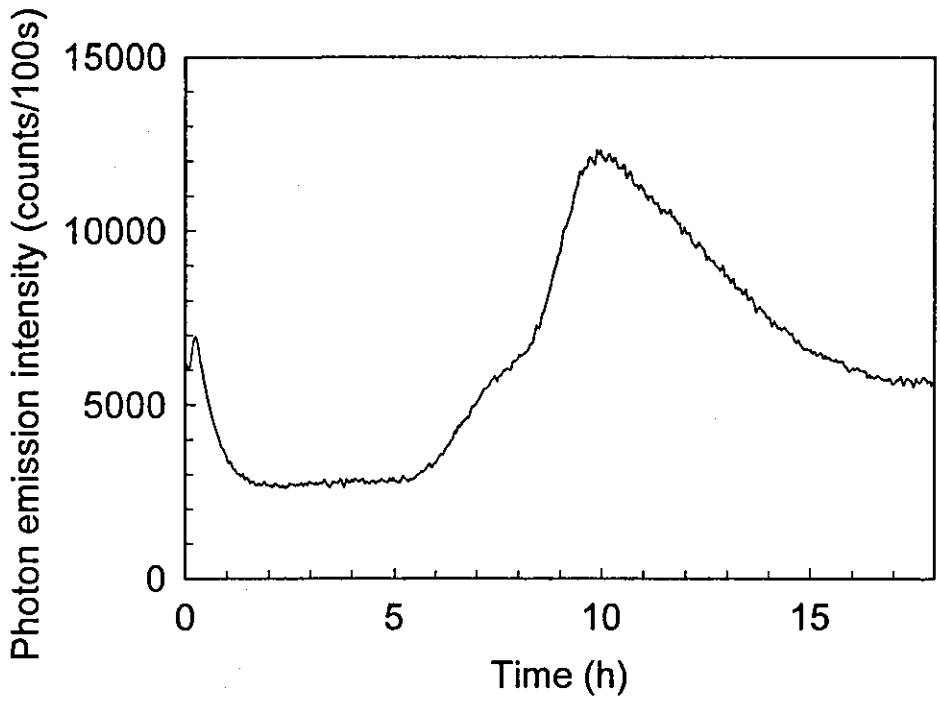

Fig. 15. A typical example of temporal changes in the intensity of biophoton emission of *Dictyostelium discoideum* during development.

characteristics of Fano factors in the period within the first 3 hours after starvation. The calculation was made with adequate time resolution with determination from the power spectral distribution of the emission intensity. Figure 16 (b) shows a typical result for the temporal changes in the Fano factor characteristics in the time region corresponding to Fig. 16 (a). The characteristic pattern indicating super-Poisson statistics in the time region around 100-150 minutes was significant in comparison with the other regions. It was compared with the Fano factor obtained from an LED, which was driven by a computer-controlled current generator to exhibit the same intensity function with time of *D. discoideum* photon emission. The intensity function in time was defined by the moving average of photocounts indicated as a solid line in Fig. 16 (a). The Fano factor characteristics of the LED are shown in Fig. 16 (c) indicating the Poisson statistics. The super-Poisson properties of Fano factor obtained after the first peak were reproduced in five individual experiments under the same conditions, with different pattern in Fano factor characteristics with observation time. The behavior of super-Poisson statistics in the particular time range is interpreted as a reflection of flash-like emission with a clustering of excitation process. Although the temporal increase of the intensity after starvation is deduced as originating from the stress-induced ROS as the biological trigger signal for development, the Poissonian behavior of photon statistics implies an intercellular independence for activation by nutritional stress. In contrast, the super-Poisson statistics obtained after the decay of the emission peak might be associated with a flash-like photon emission caused by cooperative process for the excitation. Although details of the biological mechanism are not clarified yet, this mechanism is interpreted as derived from a cooperative system among cells that can be described with synchronous activation and suppression in biological

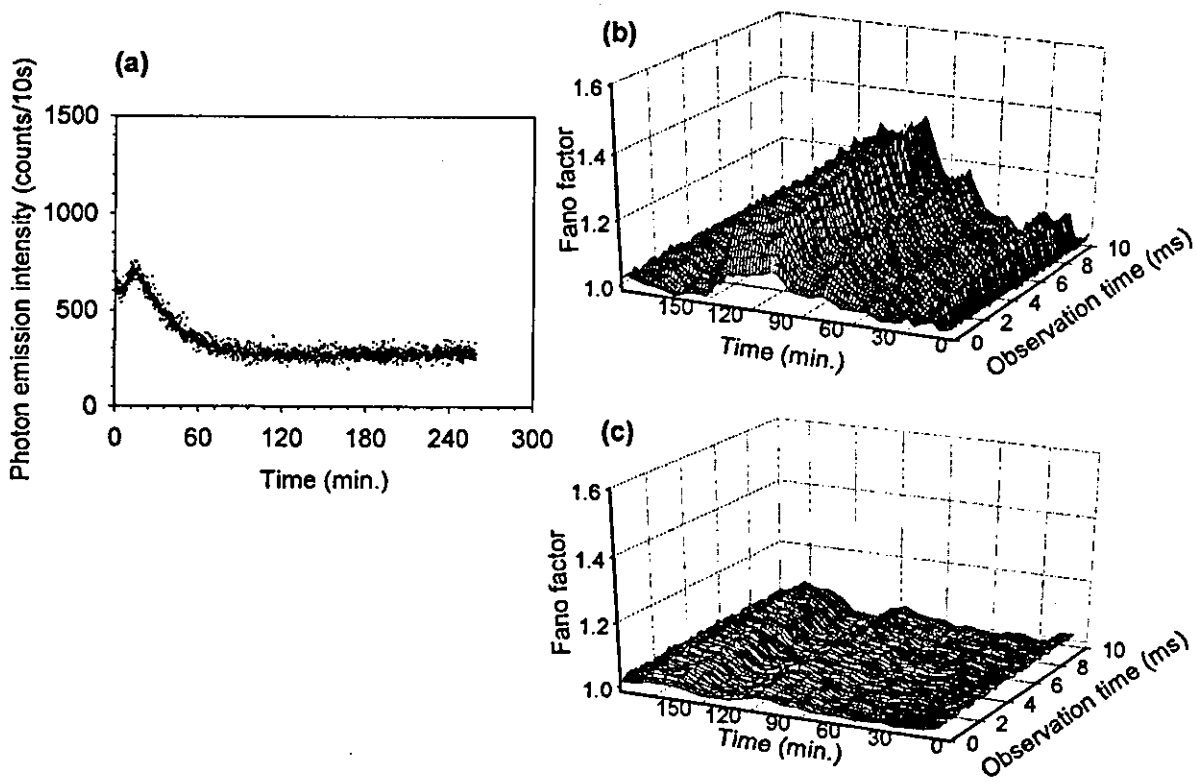

Fig.16. (a) Temporal increase of the biophoton emission intensity of *Dictyostelium discoideum* after starvation. The dots show raw data calculated with an observation time of 10 s, and the solid line shows the result of its moving average. (b) Temporal variation of the Fano factor characteristics with observation time of biophoton emission from *Dictyostelium discoideum* during the early stage of development, corresponding to the time range shown in Fig. 16 (a). (c) Temporal variation of the Fano factor characteristics obtained from an LED, which was driven to exhibit the same intensity time course as *D. discoideum*, as indicated by the solid line of Fig. 16 (a).

signaling.

When a clustering of excitation and emission processes is assumed with an optical field composed of a sequence of independent flashes of chaotic light, which is initiated by Poisson random time events,[29] the Fano factor characteristic during the observation time is theoretically described [30]. The characteristic decay time of the individual flash event of excitation were estimated 10 ms or 3 ms depending on each experiment.

Time course of $[Ca^{2+}]_i$ that had been reported is quite similar to that of biophoton emission intensity with suggesting the relationship between gene expression triggered by Ca^{2+} signal and activation (or perturbation) of cellular metabolism which derives the enhancement of biophoton emission [31]. The temporal increase of $[Ca^{2+}]_i$ after starvation is also as important signal to control the differentiation [32]. The correspondence of the pattern of augmentation observed with biophoton suggests the presence of the same mechanism. Spatially localized spike-like emissions of $[Ca^{2+}]_i$, which were interpreted as signal transduction through localized intercellular cooperation, were also reported [31, 33]; hence our observation of biophoton as super-Poisson statistics, or photon clustering, is inferred derived from Ca^{2+} spikes. The super-Poisson characteristics may reflect the properties on elemental processes of photon

emission that constitute the Ca^{2+} spike. Single cells that simultaneously respond to the localized signal for $[Ca^{2+}]_i$ release, which could be triggered by cAMP (adenosine 3', 5'-monophosphate); might contribute to the Fano factor characteristics with bearing fast decay time.

5. CONCLUSION

In this review, our studies focused on techniques and instrumentation for highly sensitive and precise determination of biophotons and their applications, investigated from various viewpoints, are discussed. Applications based on these techniques are introduced over a range of subjects, from the level of microbes to that of humans. Although the detailed mechanisms of biophoton emission in each case have not yet been clarified, the experimental results introduced here show a high potential for analyzing biophotons for the quantification of physiological and pathological information. The most valuable merit of analyzing biophotons is that it is apparently a non-invasive technique, and it cannot be influenced in the same way as other methods that use extrinsic physical probes. However, the weakness of the intensity of biophoton, which may restrict its application, requires a breakthrough in analytical technique. In that sense, I believe that our technologies for the physical analysis of biophotons could form the basis for a novel stage in biophoton research. In particular, the statistical characterization of biophotons with spatiotemporal determination will provide a novel approach by which biological order emerged with the interaction of biological reactions can be characterized, even when the photon density is very low.

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