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Multi-Site Recording and Spectral Analysis of Spontaneous Photon Emission from Human Body

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Key Words
Ultraweak photon emission · Delayed luminescence · Body symmetry · Emission spectrum · Free radicals · Lipid peroxidation · Blood flow

Summary
Background: In the past years, research on ultraweak photon emission (UPE) from human body has increased for isolated cells and tissues. However, there are only limited data on UPE from the whole body, in particular from the hands. **Objective:** To describe a protocol for the management of subjects that (1) avoids interference with light-induced long-term delayed luminescence, and (2) includes the time slots for recording photon emission. **Material and Methods:** The protocol was utilised for multi-site recording of 4 subjects at different times of the day and different seasons, and for one subject to complete spectral analysis of emission from different body locations. An especially selected low-noise end-window photomultiplier was utilised for the detection of ultraviolet / visible light (200–650 nm) photon emission. For multi-site recording it was manipulated in three directions in a darkroom with a very low count rate. A series of cut-off filters was used for spectral analysis of UPE. 29 body sites were selected such that the distribution in UPE could be studied as right-left symmetry, dorsal-ventral symmetry, and the ratio between the central body part and extremities. **Results:** Generally, the fluctuation in photon counts over the body was lower in the morning than in the afternoon. The thorax-abdomen region emitted lowest and most constantly. The upper extremities and the head region emitted most and increasingly over the day. Spectral analysis of low, intermediate and high emission from the superior frontal part of the right leg, the forehead and the palms in the sensitivity range of the photomultiplier showed the major spontaneous emission at 470–570 nm. The central palm area of hand emission showed a larger contribution of the 420–470 nm range in the spectrum of spontaneous emission from the hand in autumn/winter. The spectrum of delayed luminescence from the hand showed major emission in the same range as spontaneous emission. **Conclusion:** Examples of multi-site UPE recordings and spectral analysis revealed individual patterns and dynamics of spontaneous UPE over the body, and spectral differences over the body. The spectral data suggest that measurements might well provide quantitative data on the individual pattern of peroxidative and anti-oxidative processes in vivo. We expect that the measurements provide physiological information that can be useful in clinical examination.

Schlüsselwörter
Ultraschwache Lichtstrahlung · Verspätete Lumineszenz · Körpersymmetrie · Emissionsspektrum · Freie Radikale · Lipidoxidation · Blutfluss

Zusammenfassung
Hintergrund: Die Forschung über ultraschwache Photonenemission (UPE) aus dem menschlichen Körper hat in den letzten Jahren im Bereich isolierter Zell- und Gewebekulturen zugenommen. Es gibt aber nur begrenzt Daten über UPE vom gesamten Körper, speziell von den Händen. **Zielsetzung:** Die Beschreibung eines Protokolls für den Umgang mit Versuchspersonen, das (1) die Interferenz der körpereigenen Biophotonenemission mit Licht induzierter, lang wirkender, verspäteter Lumineszenz vermeidet, und (2) genügend Zeit für das Erfassen der Photonenemission lässt. **Material und Methoden:** Dieses Protokoll wurde zur Erfassung der Emission an verschiedenen Körperstellen von 4 Personen zu unterschiedlichen Tages- und Jahreszeiten eingesetzt. Bei einer Person wurde eine Spektralanalyse der Emissionen von verschiedenen Körperstellen durchgeführt. Ein speziell ausgewählter Photoverstärker mit rauscharmem Endfenster wurde für die Erfassung der Photonenemission im Bereich des ultravioletten und sichtbaren Lichts eingesetzt (200–650 nm). Um verschiedene Körperstellen zu vermessen, wurde der Photoverstärker in drei Richtungen in einer vollkommenen Dunkelkammer mit sehr niedriger Basiszählrate bewegt. Eine Reihe von Cut-off-Filtern wurde für die Spektralanalyse der UPE eingesetzt. 29 Körperstellen wurden so ausgewählt, dass die Links-Rechts-Symmetrie der UPE, die Symmetrie zwischen dorsalen und ventralen Körperbereichen und das Verhältnis der UPE zwischen zentralen Körperteilen und Gliedmassen untersucht werden konnten. **Ergebnisse:** Die Schwankung der Photonenzählraten über den Körper war im Allgemeinen morgens niedriger als nachmittags. Emissionen aus der Thorax-Abdomen-Region waren am schwächsten und am konstantesten. Die oberen Extremitäten und die Kopfregion emittierten am meisten Photonen und im Laufe des Tages zunehmend. Die Spektralanalyse der schwachen, mittleren und starken Emissionen von den oberen frontalen Teilen des rechtens Beins, der Stirn und beiden Handflächen innerhalb des Sensitivitätsbereichs des Photoverstärkers zeigte eine relativ grosse spontane Emission bei 470–570 nm. Die inneren Handflächen zeigten einen relativ gesehen grösseren Beitrag im Bereich des Spektrums zwischen 420 und 470 nm der spontanen Emission der Hand, und dies vor allem im Herbst/Winter. Das Spektrum der verzögerten Lumineszenz von der Hand zeigte die Emission im gleichen Bereich wie die spontane Emission. **Schlussfolgerungen:** Beispiele von UPE-Erfassung an verschiedenen Körperstellen und die Spektralanalyse zeigten individuelle Muster und Dynamiken der spontanen UPE über den Körper und spektrale Unterschiede über den gesamten Körper. Die spektralen Daten legen nahe, dass Messungen quantitative Daten über individuelle Muster peroxidativer und antioxidativer Prozesse bei einer lebenden Person liefern. Wir erwarten, dass die Messungen physiologische Informationen liefern, die auch bei klinischen Untersuchungen wertvoll sein könnten.

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Introduction

Since the 1970's, several researchers have studied spontaneous, ultraweak light emission (UPE, biophotons) from the human body [cf. 1]. Earlier work documented that emission is detectable from the upper portion of a subject standing in a darkroom in front of a photomultiplier tube. The emission was approximately 10% above background [2]. The signal-noise ratio was improved by cooling the photomultiplier, consequently, photon count rates of very few photons / ($s \times cm^2$) and depending on body location could be achieved [3]. Such low values corresponded with earlier publications on UPE from specific isolated animal tissues and organs [4–7]. Recording of UPE directly from the body was non-invasive providing continuous and convenient monitoring. This method became a unique technique for routine application in the laboratory, especially to detect peroxidative processes and the effectiveness of antioxidants for the human body in vivo [8, 9].

Multi-site recording of spontaneous UPE from the human body was achieved with a special device that allowed to manipulate a highly cooled photomultiplier tube over the human body in 3 directions [10]. Photon emission from the hands of one subject was recorded daily over more than a 9-month period [11, 12]. Data provided evidence that photon emission from both hands was usually identical and fluctuated over weeks or even months. Spontaneous emission from the hands was generally higher in summer than in autumn, winter and spring. Summer-winter differential was later confirmed in more subjects [13]. In vivo wavelength spectrum is commonly used to identify the source of spontaneous photon emission. For several isolated mammalian tissues and cells, various emission bands in the range of 400–720 nm have been described for singlet molecular oxygen and excited carbonyl groups [6, 7, 14]. The construction and high sensitivity of the moveable photomultiplier allows systematic scanning and spectral analysis of photon density of the human body. The moveable photomultiplier can yield reliable data if the following methodological questions have been answered: What time is required, after entering the darkroom, until a reliable level of spontaneous emission can be recorded? How stable is spontaneous photon emission at a given body location? What is the minimum recording time that preserves valid data averaging at a given location? Does the pattern change during the day? Does photon emission over different body locations reflect a symmetrical pattern?

Analysis of the wavelength spectrum of UPE in the ultraviolet (UV) and visible light range (200–650 nm) addresses high, intermediate and low emissions. Recording locations were selected based on multi-site recording data. In addition, the wavelength spectra of emission from hands were compared for seasonal effects in summer and autumn as were the spectrum of spontaneous emission and the spectrum of delayed luminescence of the same hand. This article presents basic findings on the applicability of this device for multi-site registration of spontaneous photon emission and discusses differences in the spectrum with regard to the pitfalls of this type of analysis.

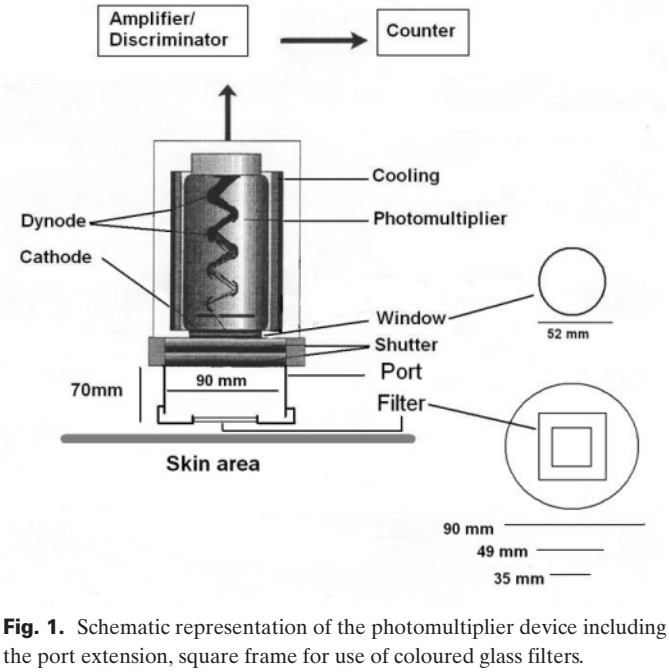

Fig. 1. Schematic representation of the photomultiplier device including the port extension, square frame for use of coloured glass filters.

Material and Methods

Subjects

Subjects were four fellow scientists: A (male, age 36), B (female, age 42), C (male, age 49), and D (male, age 61). The four subjects were self-reportedly healthy and non-smokers.

Darkroom and Photomultiplier Device

A special darkroom was utilised for the detection system with a door to a control room, located in juxtaposition to the darkroom. The control room housed the computer system. The darkroom was not exposed to light sources; its walls and ceiling were covered with black matt paint. The inner size of the darkroom was $2 \times 1.5 \times 2$ m and it had an average temperature of 20 °C. The room could be vented, the resulting small fluctuations in room temperature gave a negligible change in dark current (electronic noise) of the photon-counting device. In the darkroom there was a bed. Subjects were measured in lying or sitting position. The maximum measurement cycle inside the darkroom lasted 90 min, unless stated otherwise.

The photomultiplier (EMI 9235 QB, selected type) with a spectral sensitivity range of 200–650 nm was designed for manipulation in 3 directions under the conditions of a darkroom. It was mounted in a sealed housing under vacuum with a 52-mm diameter quartz window. It was maintained at a temperature of –25 °C to reduce the dark current. The dark current was measured weekly, at least before and after each experiment. During the experimental period the average background ranged between 4.8 ± 0.11 cps (counts per second) and 5.5 ± 0.12 cps. A spacer (a ring 70 mm high) at the front port of the photomultiplier tube allowed the measurement of a 90-mm diameter body area at a fixed distance (fig. 1). The front ring was vented inside to avoid condensation of moisture on the quartz window.

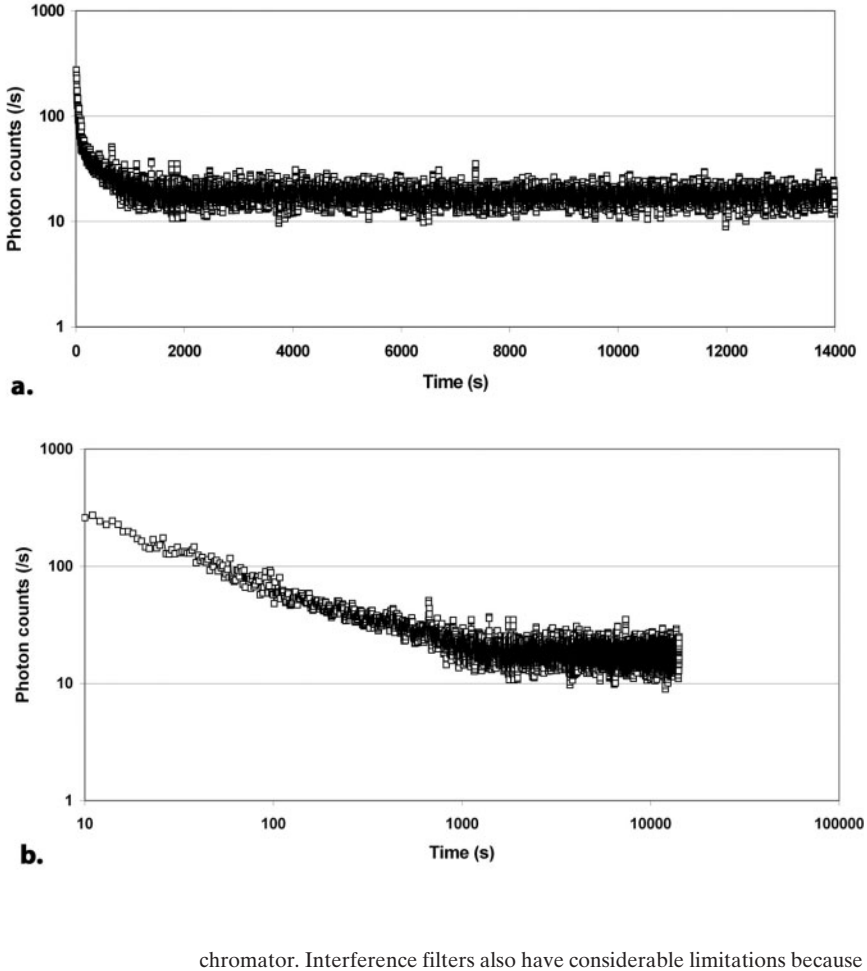

Fig. 2. Photon emission decay kinetics of the hand (dorsal left) of subject A following irradiation with artificial sunlight, **a** presented as a log photon emission vs. time plot, **b** presented as a log-log photon emission vs. log time plot.

Recording of Spontaneous Emission

Body areas were shielded from ambient light for at least 1 h before measurement. During that time the subject remained in the red dim lit control room. The subject then entered the darkroom and was positioned there for at least 10 min. Subsequently, the photomultiplier tube was placed above the body, the ring of the front port touching the body. Each recording lasted 2 min, consisting of 120 intervals of 1 s, unless otherwise stated.

Photo-Induction of Delayed Luminescence from the Hand

An Osram Ultra Vitalux 300 W (Osram, Munich, Germany) daylight lamp was used as illumination source, with a spectral range from 280 to 1,800 nm. Spectral radiation of that lamp was adjusted to solar daylight, where 16 lamps per m^2 at a distance of about 50 cm between the bulb and the irradiated object yield approximately 1 kW/m^2, which corresponds to the radiant power of the sun on the surface of the earth at noon of a summer day. Delayed luminescence was induced by illumination of the hand (dorsal) with one lamp for 2 min at a distance of 20 cm in the control room. The subject entered the dark room immediately after illumination and was positioned in the measuring position. The mean time between the end of excitation and the beginning of the measurement was approximately 7 s. This was the time required to put the subject's hand into the measurement position and then open the shutter.

Spectral Analysis of Spontaneous Emission and Delayed Luminescence

Prior to measurement, the subject was shielded from ambient light for at least 1 h. The subject was then positioned in the darkroom in lying position, and the tube placed for recording. Each recording of photon emission consisted of a timed series of intervals of 1 s; the total duration is stated in the results section. Glass cut-off filters were utilised in the present study. Since spontaneous human body emission is very weak, such emission cannot be captured with prism dispersion utilising a narrow monochromator. Interference filters also have considerable limitations because their transmission coefficients are usually low and their spectral accuracy is limited to radiation that arrives perpendicular to the filter plane. Therefore, cut-off glass filters with high transmittances were utilised to prevent loss of photons when recording such very weak emission. To estimate the wavelengths of emission in the UV and visible spectra, a square frame was mounted on top of the spacer extension of the photomultiplier tube (cf. fig. 1). A set of high-transparency (optical transparency ≥90%) glass filters with cut-off wavelengths at 320, 360, 420, 470, 530, 570, and 630 nm was used (Schott AG, Mainz, Germany), each mounted inside the square frame. The window size of filter mount was 49 × 49 mm, or 35 × 35 mm. The filters were checked for auto-luminescence. Auto-luminescence between the different filters fluctuated between 6.04 and 9.61 cps, including electronic noise. These values were stable over very long periods (up to 1 year) if the filters were kept in the dark. Quantum efficiencies for different spectral bands were calculated from technical specifications: 29.7% (200–320 nm), 33.6% (320–360 nm), 33.3% (360–420 nm), 29.0% (420–470 nm), 20.0% (470–530 nm), 8.9% (530–570 nm), and 3.1% (570–630 nm). For spectral analysis of delayed luminescence, luminescence was expressed as average photon emission in the period 7–240 s after illumination with the daylight lamp. Statistical analysis of photon count data was performed with Statistica 6.0.

Results

Spontaneous Photon Emission and Delayed Luminescence

Since photon emission can be a result of previous exposure to light, we made sure to avoid the latter. This is particularly

relevant for the hands and face which are frequently exposed to sunlight. For experimental purposes, delayed luminescence was registered over the palm of the hand after photo-induction via light exposure to a daylight lamp. Luminescence including dark rate was measured continuously for 4 h and later at hourly intervals for 12 h after the end of the illumination. Figure 2.a illustrates plot of intensity versus time in a single logarithmic scale. The plot deviates from a single exponential function. Instead, the half-life of fading of light-induced photon emission increased over time almost logarithmically till emission reached a constant value at about 2,500 s, which persisted then over 3 hours at a rate of 20.6 ± 0.7 cps (fig. 2.b). It must be noted that the mean (± s.e.m.) background noise was 6.0 ± 0.4 cps. This suggests that under the given experimental conditions the basic spontaneous emission of the body lies in the range of 15 cps (<1 cps/cm^2 skin surface). In additional studies, 'dark-adaptation' utilising dark red light conditions of the control room led to similar conclusions [personal observations]. Based on these studies, it is advisable that subjects be dark-adapted for 1 h in full darkness or under dark red light before recording photon emission from their bodies.

Time Slots for Recording Photon Emission

For comparative studies of spontaneous emission at different locations, a recording duration must be selected that allows both registration of as many body locations as possible within a reasonable time, and a high accuracy to reliably distinguish between intensity of different locations. These two variables, however, act in an opposite manner if the subject's time in the darkroom is limited, because high accuracy is commonly obtained with longer recording periods. Therefore, to estimate an appropriate length of recording, a representative time series of photon counts of the palm of the hand of a healthy person is recorded and analysed. As in any experimental set-up there is a certain variation in photon counts. From statistical analyses it has been concluded that recording times of 120 s, with emission counts grouped by seconds, give reliable values that can be used for further analyses.

Topographical Variation of Spontaneous Photon Emission

Figure 3 illustrates the body locations that were chosen for the recording of photon emission. The locations were selected such that the distribution in emission could be studied in terms of right-left symmetry, dorsal-ventral symmetry, and the ratio between the central body part and extremities. Emission from each location was measured for 2 min at a window diameter of 9 cm. The total measurement time was approximately 75 min. The front of the body (ventral) was recorded first, beginning at the forehead and then moving downwards toward the feet, at each level from left to right. Subsequently, the dorsal part of the body was recorded, beginning with the legs and moving upwards toward the head.

Figures 4–7 illustrate the emissions of the four subjects at different times of the day. Differences >1.1 cps between two locations were statistically significant ($p < 0.01$). The range of maxima and minima was quite different between the subjects. Comparing morning (approx. 9.45–11.15 a.m.) and afternoon (approx. 4.00–5.30 p.m.) recordings, the topological distribution remained generally constant in each individual, while in all subjects the emission increased in the afternoon, albeit to a different extent in each individual. Emission of subject B reflected a minimal difference between morning and afternoon with the least fluctuation of photon counts over the body in the morning. In all subjects, the thorax-abdomen region emitted lowest and most constantly, while the upper extremities (subjects A, B and D) and the head (subject C) emitted highest. If large fluctuations over the body occurred, right-left symmetry remained, but dorsal-ventral symmetry was not observed. From the emission from hands, forehead and upper legs of subject A it was concluded to measure the spectral distribution of these body locations.

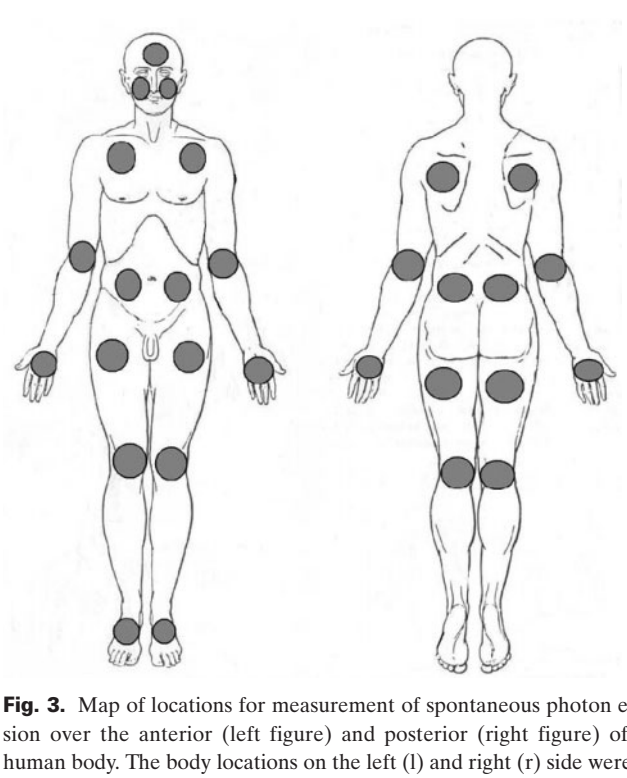

Fig. 3. Map of locations for measurement of spontaneous photon emission over the anterior (left figure) and posterior (right figure) of the human body. The body locations on the left (l) and right (r) side were abbreviated as follows: forehead (FHD), cheek (Chk), thorax-anterior (Th-A), thorax-posterior, scapulae (Th-P), abdomen-anterior (Ab-A), abdomen-posterior, kidneys (Ab-P), elbow-anterior (El-A), elbow-posterior (El-P), hand palm (Ha-p), hand dorsal (Ha-d), upper leg-anterior (Up-A), upper leg-posterior (Up-P), knee (Kne), hollow of knee (HKn), and foot frontal (Fof).

Spectral Recordings of Spontaneous Visible Emission at Different Body Locations

This procedure was based on the detection of biophoton emission in the visible and UV range within the spectral sensitivity of 200–650 nm using a series of cut-off filters with a surface of 49 mm × 49 mm. Photon emission was first recorded without and subsequently with filters in a sequence from 320 nm to

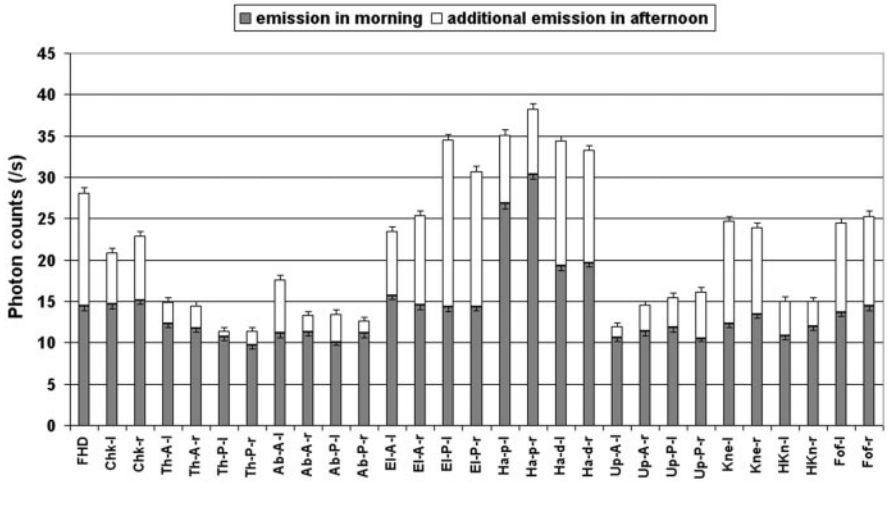

Fig. 4. Multi-site spontaneous photon emission of subject A in the morning and in the afternoon. Darkroom values before and after multi-site recording were 6.0 ± 0.4 cps and 6.4 ± 0.4 cps, respectively. The latter data include mean dark current of the photomultiplier of 4.7 ± 0.3 cps. For abbreviations, see fig. 3.

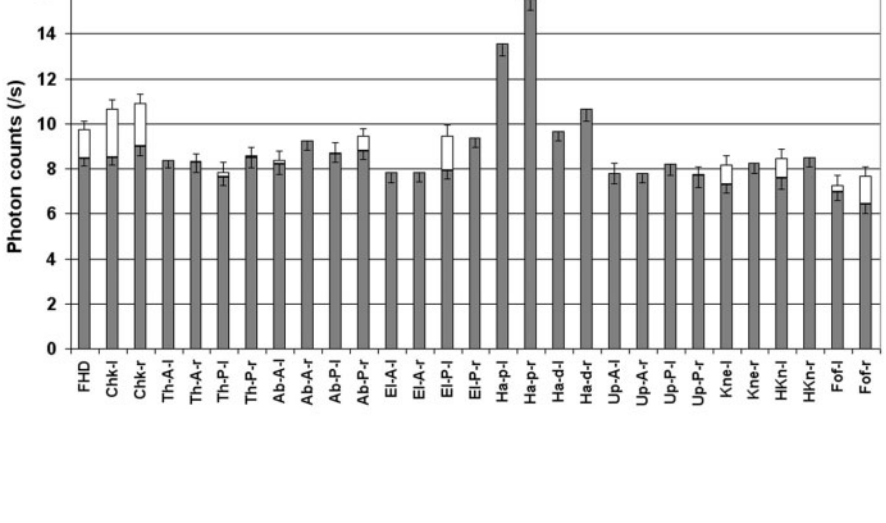

Fig. 5. Multi-site spontaneous photon emission of subject B in the morning and in the afternoon. Darkroom values before and after multi-site recording were 5.9 ± 0.3 cps and 6.2 ± 0.4 cps, respectively. The latter data include mean dark current of the photomultiplier of 5.0 ± 0.4 cps. For abbreviations, see fig. 3.

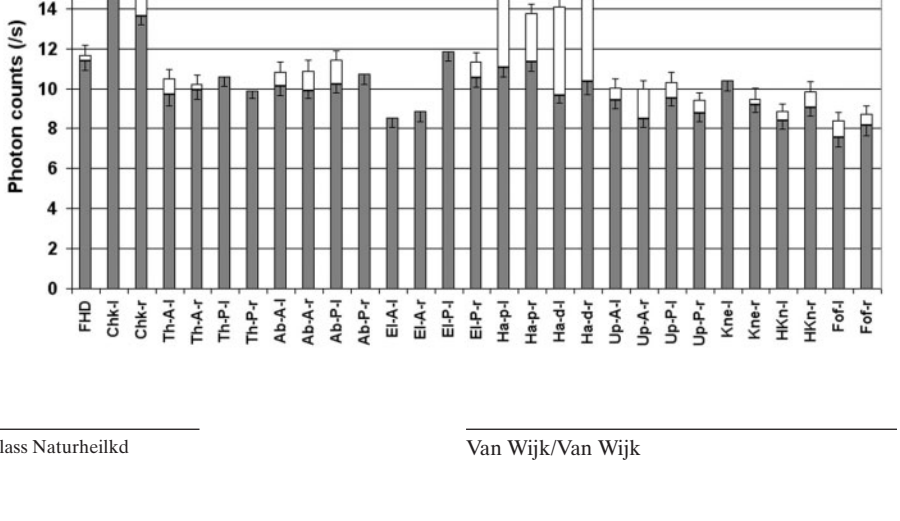

Fig. 6. Multi-site spontaneous photon emission of subject C in the morning and in the afternoon. Darkroom values before and after multi-site recording were 6.2 ± 0.4 cps and 5.8 ± 0.4 cps, respectively. The latter data include mean dark current of the photomultiplier of 5.3 ± 0.4 cps. For abbreviations, see fig, 3.

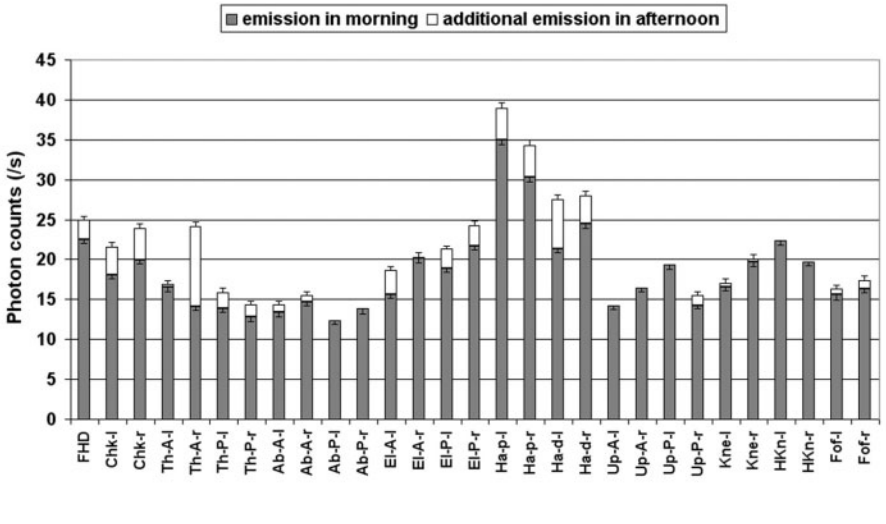

Fig. 7. Multi-site spontaneous photon emission of subject D in the morning and in the afternoon. Darkroom values before and after multi-site recording were 6.1 ± 0.4 cps and 5.6 ± 0.3 cps, respectively. The latter data include mean dark current of the photomultiplier of 4.4 ± 0.3 cps. For abbreviations, see fig. 3.

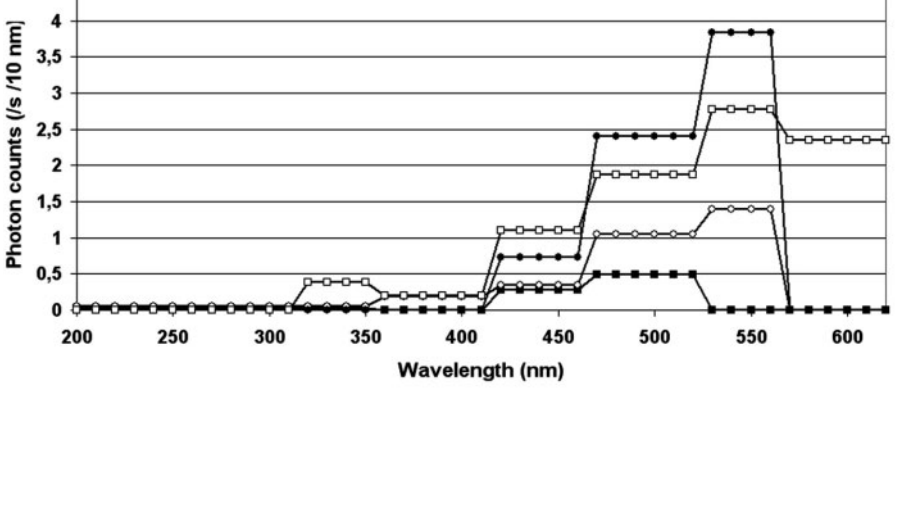

Fig. 8. Spectral distribution of spontaneous emission of different body sites of subject A: (■), superior frontal part of the right leg (upper right leg); (□), forehead; (●), left hand; (○), right hand.

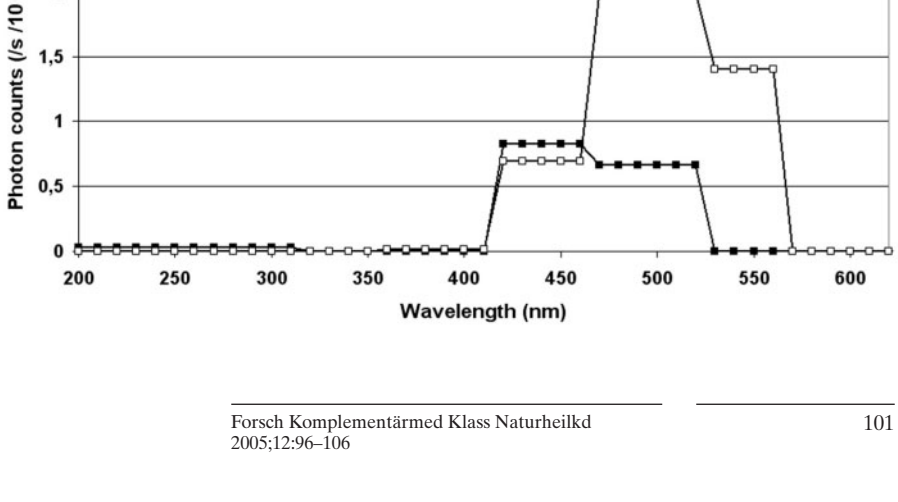

Fig. 9. Spectral distribution of spontaneous emission of the left hand of subject A during high-intensity (□) and low-intensity (■) period.

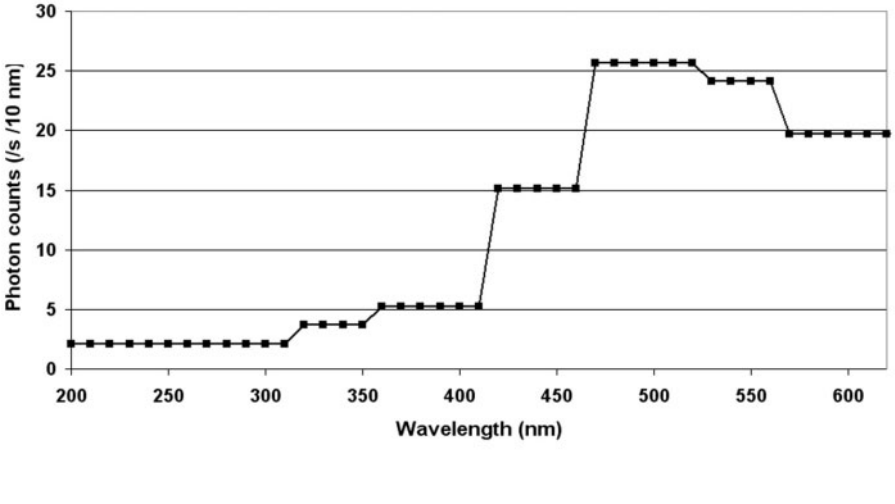

Fig. 10. Spectral distribution of light-induced delayed luminescence of the left hand of subject A hand during low-intensity period.

Table 1. Spontaneous emission of superior frontal part of the right upper leg, forehead, left hand and right hand of subject A with different cut-off filters, and emission of filters alone

Cut-off wave-length, nm	Filter alone, cps	Upper leg (right), cps	p[1]	Forehead, cps	p	Left hand, cps	p	Right hand, cps	p
No	5.4 ± 0.12	6.4 ± 0.11	0.0000	8.5 ± 0.12	0.0000	10.7 ± 0.13	0.0000	11.8 ± 0.13	0.0000
320	9.6 ± 0.14	10.5 ± 0.15	0.0000	12.3 ± 0.15	0.0000	14.6 ± 0.16	0.0000	15.6 ± 0.16	0.0000
360	6.8 ± 0.13	7.7 ± 0.13	0.0000	9.5 ± 0.14	0.0000	12.1 ± 0.14	0.0000	12.3 ± 0.15	0.0000
420	9.6 ± 0.15	10.6 ± 0.14	0.0000	11.9 ± 0.14	0.0000	14.9 ± 0.15	0.0000	14.7 ± 0.15	0.0000
470	8.2 ± 0.13	8.8 ± 0.13	0.001	10.0 ± 0.13	0.0000	12.4 ± 0.14	0.0000	11.8 ± 0.14	0.0000
530	6.4 ± 0.12	6.5 ± 0.14	n.s.	6.9 ± 0.12	0.002	7.7 ± 0.14	0.0000	7.8 ± 0.13	0.0000
570	6.1 ± 0.13	5.9 ± 0.12	n.s.	6.1 ± 0.12	n.s.	6.1 ± 0.12	n.s.	6.5 ± 0.12	0.02
630	6.1 ± 0.12	5.9 ± 0.13	n.s.	5.7 ± 0.13	n.s.	5.7 ± 0.12	n.s.	6.1 ± 0.12	n.s.

[1]Statistical significance of the difference (skin + filter) – filter.
n.s. = Not significant, i.e. $p > 0.05$; 0.0000 for $p < 0.00005$.

630 nm. Immediately after this series, a second, third and fourth identical sequence were recorded. Finally, another recording without filter completed the series of each subject. The recording time for each filter was 5 min at intervals of 1 s. Each recording with the full set of filter conditions took approximately 45 min. This afforded a comparison of emission with the same filter over 4 separate sequences within a 3-h period, to determine the stability of photon emission over 3 hours.

Four body areas of subject A were selected for this analysis: superior frontal part of the right leg, forehead, and palms of left and right hand. Although emission at each body area can increase during the day, the different areas continued to have the same relationship with the highest emission from the hands, the lowest from the upper leg, and intermediate values from the forehead (table 1). When emission values recorded without filter were corrected for electronic noise (4.8 ± 0.11 cps), emission intensities were 5.9, 7.0, 3.6, and 1.6 for left hand, right hand, forehead and upper leg, respectively. These data already demonstrate the variability of the emission over the body of one subject when recording within the full spectral range. Table 1 gives averages recorded under each filter condition. In general, emission was lower when the cut-off tended toward the red part of the spectrum. The decrease was not fully consistent, though. Some filters demonstrated a slightly higher emission than the emission recorded with the previous filter (with cut-off at shorter frequency) or when no filter was present. This reflects the differences in auto-luminescence of the individual filters (column 2) together with the inevitable physical statistics of the processes involved. In the subsequent calculations, each filter value was corrected for its own dark reading. The difference [(skin + filter) – filter] for each corresponding filter was tested for its statistical significance. Highly significant body emission could be detected with

cut-off filters ranging up to 470 nm at the upper leg, up to 530 nm at the forehead and left hand, and up to 570 nm at the right hand. If the difference [(skin + filter) – filter] was not statistically significant ($p > 0.05$), emission of the body with that filter was considered as zero. If a significant body emission was detected, the difference in body emission between two filters with successive wavelength cut-offs was used to calculate the emission of a particular wavelength range. The differences between filters with successive wavelength cut-offs were then combined with the technical specifications of the transparency of the filters and the quantum efficiency of the photomultiplier tube. The final estimation of the spectral distribution of the four body locations was expressed in cps per 10 nm (fig. 8). Emission was not present in the 200–320 nm range at any of the emission sites.

At low-emission body sites, emission was largely in the 420–530 nm range. For both hands and the forehead, the spectrum was extended particularly in the red part. This broadening is most likely due to the increased probability of differentiating between emission values with subsequent filters. However, there is also some indication that specific spectral differences are present between two symmetrical corresponding body sites, particularly with respect to both hands. The percentage of emission recorded with a cut-off filter of 470 nm was 57% and 83% of the total emission for the right and left hand, respectively.

Spectral Analysis of the Hand in High- and Low-Emission Periods

As previous studies reported high emission values in summer (from now on called high-emission period) and low values in autumn, winter and spring (low-emission period) [11, 13], we studied emissions from the hand at different times of the year. The 5 recordings in low-emission periods were carried out on consecutive Tuesday mornings in October and November 2002. The 4 recordings in high-emission periods were carried out on consecutive Tuesday mornings between mid-July and mid-August 2002. We could confirm emission differences between these periods. Table 2 gives emission levels in low- and high-emission periods recorded with the full opening (64 cm^2) of the front port of the photomultiplier tube and without filter. Emission levels in high-emission periods were 2–3 times the emission in low-emission periods.

These recordings of the hands were also carried out utilising the series of cut-off filters in a smaller frame (35 mm × 35 mm; measured surface area: 12.25 cm^2) to estimate the spectrum of the emitted light. In the low-emission period, 5 spectral analyses were achieved on 5 different days with 5-min measurement periods. In the high-emission period, 4 spectral analyses on 4 different days were achieved with 3-min measurement periods. The results recorded on different days with an individual filter were averaged (table 3). In the high-emission period, emission was no longer detectable with cut-off filters at 570 nm and higher. In the low-emission period, the increased measurement time resulted in smaller standard error values and emission was no longer detectable with cut-off filters at 530 nm and higher. The spectral distribution of the hand in the high- and low-emission periods was calculated as described above. The data allowed the recording of emission in the 420–570 nm range (fig. 9) in the high-emission period. The spectral distribution of the hand in the low-emission period shows no emission in the 530–570 nm range contrary to the high-emission recording. Neither in low- nor in high-emission periods, UPE was detected in the 200–420 nm range.

Table 2. Spontaneous emission of the hand of subject A

Experiment	Low-emission period, cps		High-emission period, cps	
	Hand[1]	Background	Hand[1]	Background
1	15.0 ± 0.28	5.1 ± 0.20	28.3 ± 0.51	5.2 ± 0.19
2	13.2 ± 0.26	4.5 ± 0.17	32.2 ± 0.51	5.7 ± 0.21
3	13.9 ± 0.26	5.0 ± 0.19	31.4 ± 0.54	5.7 ± 0.20
4	13.7 ± 0.27	4.8 ± 0.18	27.4 ± 0.51	5.5 ± 0.20
5	14.4 ± 0.27	5.1 ± 0.19		

[1]Values include background electronic noise. Average background was 4.9 ± 0.11 and 5.5 ± 0.12 cps in low- and high-emission period, respectively.

Spectral Measurement of Light-Induced Delayed Luminescence with Coloured Filters

In the low-emission period a comparison between the spectrum of spontaneous and light-induced delayed luminescence of the hand was computed. The hand was illuminated using the daylight lamp, the resulting emission was considerably higher than spontaneous emission. Delayed luminescence was determined by counts over a period of 7–240 s, with the coloured glass filters analogously to the spectral method above. Figure 10 illustrates the emission spectrum, which should be compared to the spontaneous emission spectrum in the low-intensity period (fig. 9). The delayed induced luminescence spectrum was between 420–630 nm. The broadening was expected because emission is higher after excitation and was also detectable with cut-off filters at 530 nm and higher.

Spontaneous Emission and Blood Supply

In an attempt to investigate one physiological aspect of photon emission aetiology, we placed a tourniquet around the upper arm to depress the supply of oxygen and nutrients to the hand. Photon counts were recorded in high-emission period, with the use of a port extension with full opening. Photon emission was calculated for the periods 2 min prior to tourniquet application, 2 min during application, at 5 min after removal of the tourniquet, each with an increasing degree of tourniquet tightness (table 4). The mean photon emission from the hand significantly and progressively decreased over

Table 3. Spontaneous emission of the hand of subject A during high- and low-emission period with different cut-off filters

Cut-off wavelength, nm	Low-emission period			High-emission period		
	hand + filter, cps	filter, cps	p[1]	hand + filter, cps	filter, cps	p[1]
no	7.3 ± 0.12	5.3 ± 0.12	0.0000	10.1 ± 0.20	6.15 ± 0.18	0.0000
320	13.0 ± 0.13	11.1 ± 0.13	0.0000	15.3 ± 0.23	11.2 ± 0.19	0.0000
360	10.4 ± 0.13	8.5 ± 0.12	0.0000	12.4 ± 0.22	8.3 ± 0.18	0.0000
420	13.7 ± 0.13	11.7 ± 0.13	0.0000	14.9 ± 0.22	11.0 ± 0.20	0.0000
470	10.8 ± 0.12	10.0 ± 0.13	0.0000	12.8 ± 0.21	9.9 ± 0.18	0.0000
530	7.7 ± 0.11	7.8 ± 0.12	n.s.	8.2 ± 0.17	7.7 ± 0.19	0.0320
570	7.1 ± 0.12	7.3 ± 0.11	n.s.	7.3 ± 0.16	7.4 ± 0.20	n.s.
630	6.8 ± 0.11	7.2 ± 0.12	n.s.	7.3 ± 0.18	7.2 ± 0.18	n.s.
695	6.9 ± 0.11	7.0 ± 0.12	n.s.	7.0 ± 0.16	7.1 ± 0.19	n.s.

[1]Statistical significance of the difference (hand + filter) – filter.
n.s. = Not significant, i.e. $p > 0.05$; 0.0000 for $p < 0.00005$.

Table 4. Spontaneous photon emission during blood flow restriction with a tourniquet

Condition	Before tourniquet, cps	p (before-during)	During tourniquet, cps	p (during-after)	After tourniquet, cps	p (before-after)
Mildly	26.7 ± 0.61	0.0500	24.5 ± 0.75	0.2400	25.7 ± 0.58	0.4100
Tight	26.2 ± 0.61	0.0000	21.8 ± 0.68	0.0000	25.7 ± 0.56	0.5700
Severely	30.3 ± 0.65	0.0000	23.4 ± 0.68	0.0038	25.9 ± 0.55	0.0000

the series. Removing the tourniquet after a mild and tight restriction resulted in a return of the photon emission rate to its former level within 5 min. After a severe restriction, recovery was slower.

Discussion

Weak photon emission from living mammal (including human) tissues was previously reviewed [15–19] with emphasis primarily on nerve and muscle tissue [20–24], isolated liver [4–5], and intact body, primarily the hand [1–3, 8, 9, 11–13, 25, 26]. Some of those previous studies addressed different body locations of the same subject. In most of those studies, procedures were either poorly described or recordings were performed utilising devices with low signal-noise ratio. This article focused on several approaches to measure the dynamics and the spectral emission from several body parts.

Although delayed luminescence of human body has previously been demonstrated [11–13, 25], its decay, particularly after excitation with (artificial) sunlight has not been studied before. In the present study, we found that illumination with artificial sunlight led to a delayed luminescence that was not represented by a simple exponential decay curve. Instead, the half-life of the fading of light-induced photon emission increased almost logarithmically over time until the emission reached a constant value. On the basis of these decay kinetics and experiences with long-term dark-adapted body, we recommend to record the body only after it has been dark-adapted for at least 1 h, otherwise external conditions can easily interfere with the recording of spontaneous emission.

Our protocol for recording body photon emission over several body parts was based on a highly sensitive conventional photomultiplier which was first used by Cohen and Popp [11, 12]. They reported that emission from local body areas is generally stable in a daily experimentation period of several hours. The present study determined more accurately the recording time necessary to obtain a reliable average value for emission with this device. This part of the study documented large variations in photon count time series at small time frames. The origin of such fluctuations is still unclear; they may have a biological origin and need further characterisation. As a consequence, with the given experimental set-up recording times of at least 120 s are necessary to achieve reliable count rates.

With the present protocol the topographical variation in emission of four subjects was analysed. Data demonstrated that variation in photon count over the body depended on the subject and on the time of day. Most morning values of most body locations were within a limited range of emission intensity. The range was 10–15 cps, 6.5–8.5 cps, 8–12 cps, and 12–17 cps for subjects A, B, C and D, respectively. Only few body loca-

tions had increased emission, such as hand palms of subjects A, B and D, and cheeks of subject C.

Studying emissions in the morning and in the afternoon demonstrated that the increase of emission showed different patterns. It either occurred at almost all locations (subject A), or at only a few locations (subject B). Subjects C and D showed an intermediate pattern. In many cases, a location with high-emission in the morning showed a further increase in the afternoon. In a few cases, an increased emission in the afternoon occurred at locations that had low emission in the morning without increase of emission at the body location that was specifically increased in the morning (subject B).

Although the body emission pattern was highly idiosyncratic, the patterns also shared some general features: Emission from the hands and head were commonly higher than from other body locations. In those subjects with large fluctuations over the body (A and D), higher values were also recorded for elbows, knee and feet. If large fluctuations occurred, right-left symmetry remained, but dorsal-ventral symmetry could not be observed. The latter was particularly evident for knees, hands and elbows. Interestingly, body parts that are more shaped and structured emitted more than rather unstructured, flat body parts. Highly structured parts like elbows, knees and hands also revealed characteristic differences between anterior and posterior parts. Further research is required to clarify any relation between emission and the anatomy and physiology of body parts, which suggests a connection with inhomogenities of the electrical field of the body surface (spike effect).

Notwithstanding the single-case nature of the present spectral analysis study, data reveal the capacity of the device for stepwise analysis of the spectra of human body sites. In the same instance, the experimental precautions and requirements were enlightened and the pitfalls described that might occur with such measurements. A major obstacle is the comparison of spectra of highly different UPE's, in particular in the spectral range that corresponds with low spectral sensitivity of the photomultiplier. However, the present data indicate that differences in the spectrum of the left and right hand of the same individual do occur. Whereas both hands have roughly similar UPE, the right hand emits over a broader spectral range than the left. Spectrum of emission from the hand in low- and high-emission periods can be compared only for the part of the spectral range that is reliably measured. Comparison showed that also in this case both spectra were different, showing that in summer the emission is relatively more with a cut-off filter of 470 nm compared to emission in winter. The presented data reporting high emissions in summer and low in autumn correspond with former observations of annual fluctuations in emission from hands [11, 13]. Spectral analysis of delayed luminescence also demonstrates major intensity in the 470–570 nm range, corresponding with the spontaneous emission in the high-emission period. At present, it is unclear if these changes are due to different dietary conditions according to the season, metabolic changes due to season or even 'storage' of photons in cellular compartments. Further research is needed to clarify this issue.

The spectral data suggest that the sources for emission might be different at different body locations and in season. The spectrum can be compared with known spectra from mammalian organs described in the literature. Previous extensive studies demonstrated photon emission from several organs and tissues, cells, cell homogenates, and certain well-specified chemical reactions. This fundamental analysis was almost completely carried out in the 1970's and 1980's, and reviewed by multiple authors [14–16, 27]. Experimental evidence cumulated to date indicates that the spectrum in the wavelength range of 200–650 nm corresponds to spectra obtained from carbonyl compounds in the excited singlet and triplet states as well as excited dimers (dimols) of singlet molecular oxygen. Such compounds are formed during the decomposition of lipid peroxides.

Spectral differences of photon emission might reflect different biochemical reactions. Excited carbonyl compounds (>C = O)* and dimoles O_2* ($^1\Delta_g$) are produced by dismutation of peroxy radicals and by peroxide cleavage. During decomposition (2 >CHOO → C = O + >COH + O_2), either the carbonyl group or the oxygen can be formed in electronically excited states. In vivo, emission from excited carbonyl groups can occur at wavelengths in the 400–500 nm range. In addition, excited singlet oxygen molecules can emit at approximately 780 nm. Other singlet oxygen emissions lie even further into the infrared. The excited singlet oxygen molecules may also excite a secondary emission by reacting with unsaturated lipids to produce excited carbonyl groups, which can then emit with wavelengths in the same vicinity (400–500 nm). Another possible emission arises after dimerisation of excited singlet oxygen, the dimer emitting two broad bands centred on 634 and 703 nm [28]. Excited carbonyl groups produced by the first two reactions reflect the blue-green components of the emission, while the emission from excited oxygen dimers is most compatible with the red emission. Thus, it can be argued that a higher proportion of unsaturated lipids accentuates the blue-green carbonyl emission, as this emission arises from the scavenging of singlet excited oxygen which would otherwise be available for the red (or infrared) emission.

The tourniquet experiments can be considered as a confirmation that oxygen is very likely involved in the emission from the body. Emission is suppressed during hypoxia. The data show that photon emission was 17% and 23% lower under tight and severe blood flow restriction, respectively. The data correspond with the observations of Edwards [3] that during the placement of a tourniquet, emission was about 15% lower. Similar effects have been reported from the cortex of rats. Emission was associated with cerebral blood flow; it was depressed after cardiac arrest [29, 30].

In conclusion, we have presented a few examples of the multisite photon emission recording and spectral analysis in order

to demonstrate individual body patterns in UPE, and spectral differences over the body. The measurements might well provide quantitative data on the individual pattern of peroxidative and anti-oxidative processes in vivo. We expect the physiological information of the measurements to be useful in clinical examination. Further research can now be initiated to develop clinical and diagnostic applications of these techniques in biomedicine.

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