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Technical communication

Anatomic characterization of human ultra-weak photon emission with a moveable photomultiplier and CCD imaging

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Abstract

Ultra-weak photon emission of a living system has received scientific attention because of its potential for monitoring oxidative metabolism and oxidative damage to tissues. Heretofore, most studies have focused only on the emission from hands. The data regarding emission from other anatomic locations are limited. A previous multi-anatomic site recording of four subjects quantitatively demonstrated that the emission from several corresponding anatomic locations could differ by as much as a factor of 4. The data also suggested a ''common'' anatomic emission percentage distribution pattern. This information raised the question whether such a typical anatomic percentage emission exists. The objective of the present paper is to systematically replicate the emission from identical anatomic locations to document whether the anatomic percentage distribution pattern is generic. Part 1 includes the recording of ultra-weak photon emission from one sample subject over the torso, head and upper extremities with a highly sensitive charge-coupled device (CCD). Part 2 includes the analysis of that data to select a series of anatomic locations that were subsequently studied with a group of 20 subjects utilizing a highly sensitive, cooled and moveable (in three directions) photomultiplier system. Total sum emission of all recorded anatomic locations per subject fluctuates in this study almost 5-fold between subjects. However, the contribution of each anatomic location to the total emission from each subject was approximately the same percentage for each subject and similar to the sample CCD subject. The deviation of the anatomic percentage contribution for each subject was also established. The study presents evidence that there is a ''common'' anatomic percentage distribution pattern of ultra-weak photon emission for corresponding locations from each subject.

Keywords: Biophoton; Ultra-weak photon emission; Photon counting; Human body; Photon imaging; Spatial emission pattern

1. Introduction

Optical imaging of organisms has grown into an important tool in biomedical research. Novel macroscopic photonic imaging technologies in combination with emerging data analysis provide researchers with several techniques to visualize biological processes. A recent review focused on photographic and tomographic optical imaging techniques, bioluminescence and photoacoustic tomography [1]. The whole-body imaging technology exploring weak light, spontaneously emitted from humans without any artificially induced external excitation or stimulation, has been receiving relatively less attention, probably because the underlying biochemical and biophysical processes are not yet well established. The ultra-weak spontaneous emission is commonly referred to as ''human biophoton emission''. The intensity of this emission in the range 200–650 nm is estimated to be on the order of less than $\sim 10^2$ photons/cm^2 body surface [2]. Boveris et al. characterized photon emission from a variety of mammalian organs in an in vivo investigation of the radical reactions initiated by lipid peroxidation [3]. Many other pioneering studies have suggested the potential usefulness of non-invasive monitoring of oxidative metabolism and oxidative damage to tissue [4–6].

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To study human photon emission and to clarify its basic mechanisms, one might use low-noise photomultiplier systems with high stability of the signal. Such a system exists suspended on runners in a light-tight dark room such that the detector head could be moved in three directions over a subject lying on a bed below [7]. A recent study utilizing this technology described a systematic multi-site recording of four subjects using 29 anatomic sites that were selected such that the distribution in photon emission could be studied as right–left symmetry, dorsal–ventral symmetry, and the ratio between the central part of the body and extremities [8,9]. Although data from that study demonstrated variability in patterns between subjects, some generic features were observed: (a) the thorax–abdomen region emits the lowest emission; (b) the hand and the head region emit the highest levels. The data also simultaneously suggested that a ''common anatomic human biophoton percentage distribution emission pattern'' exists corresponding to the above described levels of emission.

A second system to fundamentally characterize anatomic distribution of human ultra-weak photon emission utilizes two-dimensional imaging technology [10]. Recent developments demonstrate spontaneous ultra-weak biophoton emission from larger human anatomic sites using CCD imaging [11]. The few already published images also suggest a ''common'' anatomic human emission percentage distribution pattern.

This paper presents two sequential protocols used to register human biophoton emission patterns. The first protocol illustrates registration biophoton emission from the upper frontal torso, head, neck and upper extremities of a single subject utilizing CCD. Data obtained with the CCD illustrate the high or low emission from anatomic detection. The knowledge of such locations was subsequently utilized to select 12 anatomic spots for future recording of more subjects in a second protocol. The second protocol studied biophoton emission from those selected 12 anatomic spots from the frontal torso, head and hands of 20 healthy males. The data from this subsequent study illustrates the existence of a ''common'' human male body emission pattern amongst the simultaneous existence of individual differences. Fluctuations of emission intensity between subjects was not limited to a few body locations, but included the entire emission pattern.

2. Materials and methods

2.1. Subjects

Twenty subjects ranging in age from 20 to 65 years were, by self-report, healthy and free of medications and smoking. They were interviewed to exclude physical or emotional disorders. Written consent to participate in the study was obtained after they were thoroughly informed about the research. The emission of each subject was recorded only once.

2.2. Imaging human body with the highly sensitive charge-coupled device (CCD) camera

A cryogenically cooled CCD camera system that incorporates a CCD42-40 NIMO Back Illuminated High Performance CCD Sensor having full-frame architecture (CCD42-40, e2v technologies, UK) was used for imaging of human biophoton emission [12]. Operating temperature of the CCD sensor is -100 °C, resulting in a dark signal (electronic noise) of 0.65 e^-/pixel/h. Spectral response of the CCD ranges over 400–900 nm with quantum efficiency of >90% at the peak wavelength of 550 nm. The measurement was carried out in binning mode, resulting in the imaging format of 256×256 pixels. The magnification of the lens system for imaging torso and arms was approximately 0.03 and the magnification for imaging the face or hand was approximately 0.13. The CCD camera system was placed in a darkroom whose walls, ceiling, and floor were covered with non-fluorescent black cloth. The camera system was controlled from the laboratory located juxtaposition to the darkroom. The darkroom included a chair; the subject, after dark adaptation, was recorded in the sitting position. The duration of such measurement inside the darkroom was 30 min. Before measurement, subjects were already inside the darkroom for at least 30 min to prevent interference by delayed luminescence.

2.3. Recording human emission with the photomultiplier

The photomultiplier (9235 QB, selected type; Electron Tubes Limited, Ruislip, England; previously EMI) with a range of 200–650 nm was designed for manipulation in three directions [7,8]. It was mounted in a sealed housing under vacuum with a 52 mm diameter quartz window maintained at -25 °C to reduce the dark current. The dark current was measured before and after each experiment. During the experimental period the average background was 5.4 ± 0.3 cps (counts per second). A 7 cm long cone shaped extender is attached to the front of the photomultiplier tube allowing recording of emission from a 9 cm diameter anatomic area at a fixed distance. The front ring is vented inside, avoiding the condensation of moisture in the quartz window.

The photomultiplier was situated in a dark room juxtaposition to the control room housing the computer system. The walls and ceiling of the dark room were covered with mat black paint. The inner size of the dark room had the following dimensions: $2\ m \times 1.5\ m \times 2\ m$ with an average temperature of 20 °C. The room could be vented. A bed was positioned in the dark room.

Subjects were commonly recorded between 11 a.m. and 2 p.m. Before recording, subjects were shielded from ambient light for at least 1 h to prevent interference by delayed luminescence [8,9]. Subjects remained during this period in the red dim light of the control room. Subjects then walked into the dark room and were positioned on the bed for at least 10 min. The photomultiplier tube was placed above

the body, the ring at the front port of the photomultiplier barely touching the body. The duration of each recording was 120 s consisting of 2400 time intervals of 50 ms.

2.4. Data analysis

Statistical analysis of photon count data was performed with Statistica 6.1 (StatSoft, Tulsa, OK, version 2004).

3. Results

3.1. CCD imaging and sequential multi-site recording with a photomultiplier of a single sample subject

The present technique of human CCD imaging, developed by one of the authors (Tohoku Institute of Technology, Sendai, Japan) is able to reveal the topography of spontaneous ultra-weak photon emission of dark-adapted human subjects. In this sequential study the cryogenic cooled CCD was utilized to image the ventral and dorsal sides of the torso and upper extremities of one sample dark-adapted subject (Fig. 1, panels A, D, G). The resulting set of large anatomic CCD images of this individual subject were obtained by recording continuously for 30 min at a distance of 100 cm. The images were compared with identically sized photographs of the subject taken under weak illumination (Fig. 1, panels B, E, H). As illustrated in the ventral image of the superior part of the body, photon emission intensity around the face and neck is highest and gradually decreases over the torso and subsequently over the abdomen. There also exists a gradual decrease in intensity from the superior central torso to its lateral dimensions. Dorsally, the highest intensity was emitted from the neck. The images of arm and hand of the subject illustrate that the low intensity of the body is extended over a large part of the arm and then increases over the hand.

The head and the hand are anatomically more complicated structures. For this reason CCD images of these anatomic locations were obtained at a distance of approximately 40 cm (Fig. 2). The image of the head demonstrates that the eyes exhibit a distinctly lower intensity (Fig. 2, panels A, B). Other locations such as forehead

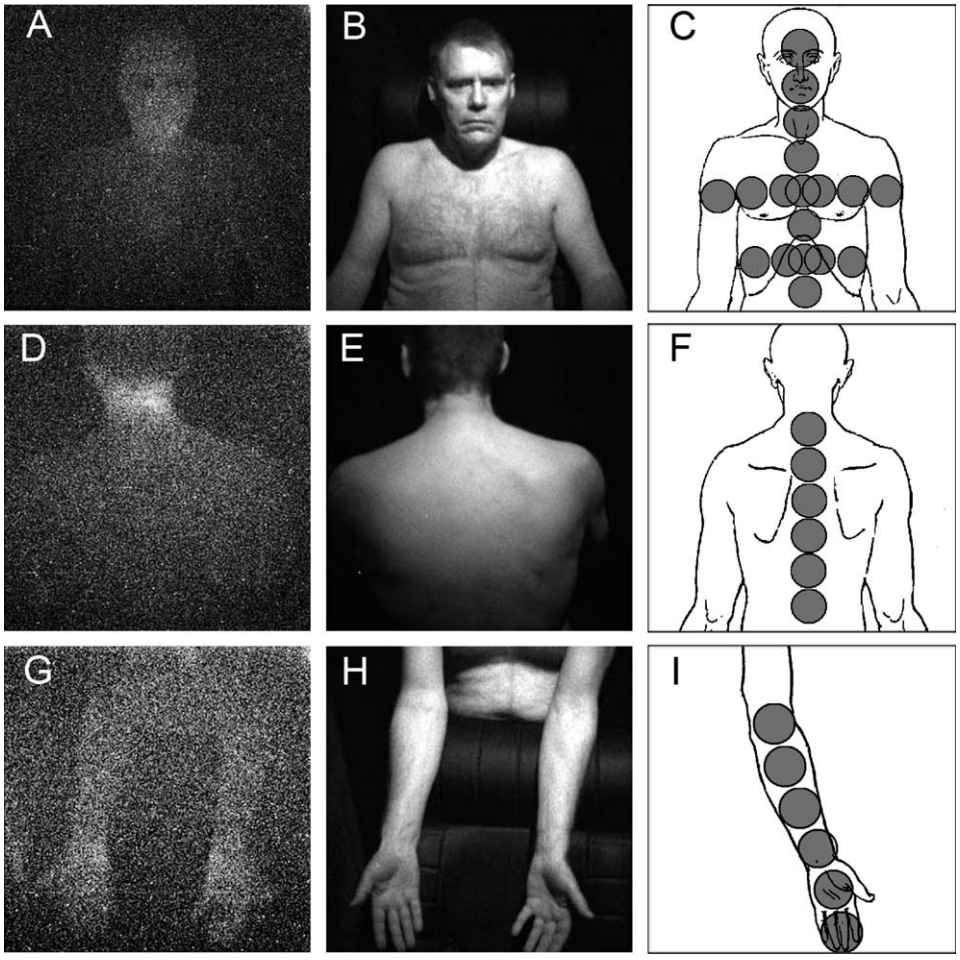

Fig. 1. Ultra-weak photon emission of the ventral and dorsal torso and arms of a human subject. Photon emission image measured with the CCD imaging system: ventral torso (A), dorsal torso (D) and arms (G). Corresponding photographs taken under weak illumination: ventral torso (B), dorsal torso (E) and arms (H). Anatomic locations used for recording of ultra-weak photon emission using the moveable photomultiplier: ventral torso (C), dorsal torso (F), arms and palms (I). Anatomic locations correspond with the respective emissions in Table 1.

and cheeks exhibit a rather homogeneous distribution of photon emission. The images of the palm and back of the hands illustrate more differentiation (Fig. 2, panels C, D). Emission between fingers fluctuates. Viewed from the palm (ventral) side, the four fingers demonstrate increased intensity from forefinger to little finger. However, the back (dorsal) side of the four fingers illustrates an increased intensity in the reverse order (little finger to forefinger). In addition, the dorsal side of the fingers and nails produce a high emission. A rather homogeneous photon emission distribution was present over the central palm and central back of the hand.

Subsequently, the locations illustrated in Fig. 1 (panels C, F, I) were used for quantitative measurements of photon emission utilizing the moveable photomultiplier device (International Institute of Biophysics, Neuss, Germany). Table 1 presents data along (a) the ventral and dorsal longitudinal axis from head to abdomen, (b) transversally from both left and right sides of the longitudinal axis, and (c) along arm and hand. The dorsal longitudinal axis illustrates lower intensities compared with the ventral. An exception is the high intensity around the neck.

The data collected transversely from both left and right anterior of the longitudinal axis were obtained from a 54 cm area over the breasts. Over the abdomen, data were reliably collected over a smaller (36 cm) transverse area. The central area has higher emission compared to the left and right sites.

Table 1
Spontaneous photon emission from body sites presented in Fig. 1

Photon emission (cps) from anatomic locations		
Location	Frontal	Dorsal
A. Longitudinal axis from head to abdomen (see Fig. 1C and F)		
Head	10.1 ± 0.3	
	13.5 ± 0.4	
Neck	14.8 ± 0.4	14.1 ± 0.3
Torso	12.5 ± 0.3	10.2 ± 0.3
	12.3 ± 0.4	8.5 ± 0.2
	11.5 ± 0.4	8.4 ± 0.3
	11.4 ± 0.4	9.0 ± 0.3
	10.8 ± 0.3	7.8 ± 0.3
Location	Breast	Abdomen
B. Transverse recordings (see Fig. 1C)		
Left-side	8.2 ± 0.4	
	9.7 ± 0.4	7.7 ± 0.3
Middle	11.9 ± 0.4	9.2 ± 0.4
	12.6 ± 0.4	9.5 ± 0.4
Right-side	9.7 ± 0.3	7.8 ± 0.3
	8.3 ± 0.3	
Location	Ventral	Dorsal
C. Locations from elbow to fingers (see Fig. 1I)		
Elbow	7.6 ± 0.3	7.1 ± 0.3
	7.5 ± 0.3	7.3 ± 0.3
	8.0 ± 0.3	7.7 ± 0.4
	9.7 ± 0.4	8.6 ± 0.4
	15.5 ± 0.4	12.1 ± 0.4
Fingers	15.6 ± 0.4	17.2 ± 0.5

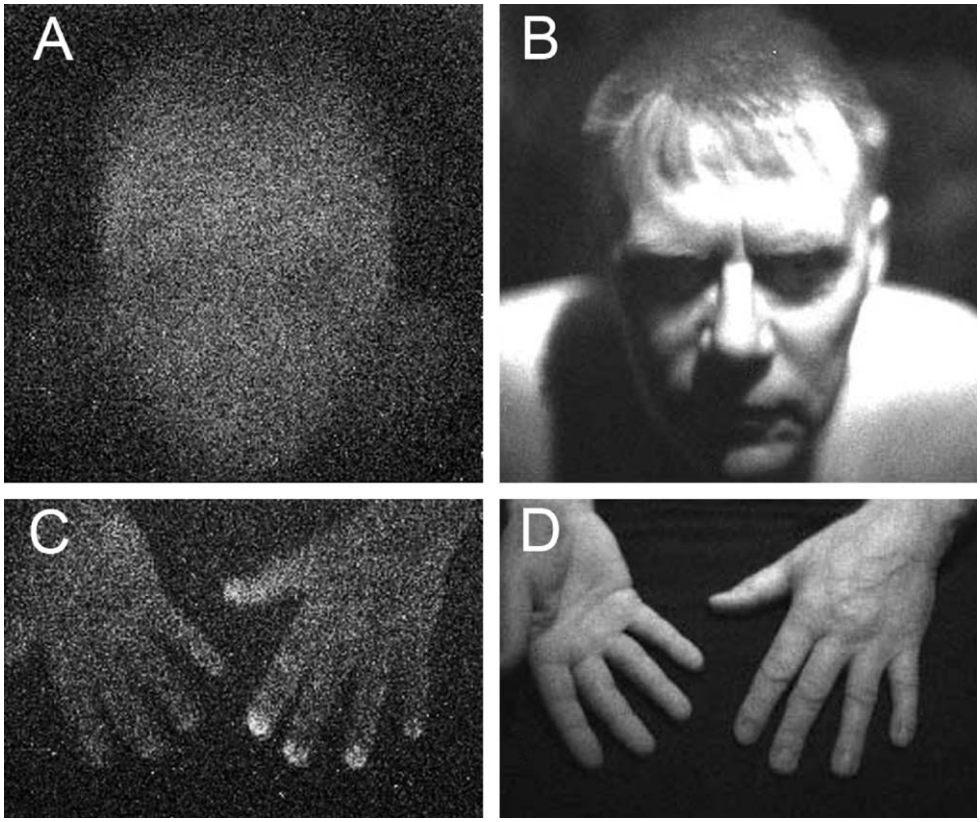

Fig. 2. Ultra-weak photon emission of the face, the palm and dorsum of the hand. Photon emission image measured with the CCD imaging system: face (panel A) and hands (panel C). The palm side is left and dorsum is right. Panels B and D are corresponding photographs under weak illumination.

The recording of photon emission along the arm and hand, both dorsally and ventrally, demonstrate that emission is low along the arm and strongly increases over the hands.

3.2. Multi-site registration of spontaneous emission from a group of male subjects utilizing the moveable photomultiplier

CCD data from the single subject described in Figs. 1 and 2 facilitated the selection of 12 full skin locations with uniform distribution of photon emission covering the wide range of intensities from low emission sites over the abdomen to high emission sites over the palms of the hand and forehead. The anatomic locations are presented in Fig. 3.

These locations were used to study whether anatomic distribution of emission reflects a "common" human anatomic percentage distribution emission pattern. It can be argued that (a) when one subject has a higher emission intensity than another subject at a specific anatomic location, it only reflects an increased emission at that location without any relationship to intensities at other locations, or (b) it reflects an increase of emission at all anatomic locations. In the latter case, each anatomic location should contribute in a proportional manner to the total emission. Therefore, the question whether there is a "common" human anatomic percentage distribution emission pattern can be answered when the emission contribution of each anatomic part of each subject to the total sum of emission is calculated for each subject.

The recordings at 12 anatomic locations were carried out with 20 control subjects. Each recording consisted of 2400 consecutive intervals of 50 ms. Recorded count values included electronic background which was subtracted in order to obtain the actual photon emission of the anatomic location. Electronic background did not change significantly during the day. However, background fluctuated during the experimental period (mid-October until mid-March) ranging between 4.9 ± 0.34 and 5.7 ± 0.42 cps (mean background 5.2 ± 0.41 cps). Thus, emission of each of the 12 anatomic locations of each subject was determined by subtracting the background value of the corresponding subject's recording session.

To estimate the contribution of each anatomic part of each subject to the total emission of that subject, total emission was defined as the sum value of the 12 recorded emissions. Fig. 4 portrays the contribution of each anatomic location to total emission for each subject.

Data demonstrate that the sum of emissions from 12 anatomic locations could differ by almost a factor of 5 between subjects; total emission can fluctuate between 51.22 and 231.97 cps. Each anatomic location's percentage emission from each subject statistically correlates ($p < 0.05$) with the total emission from each subject.

Extrapolation of each regression line approaches zero, suggesting that, in principle, each individual anatomic part participates in total emission with a constant percentage. The contribution of each anatomic location to total emission is not equal. The percentage of contribution is shown in Table 2. Photon emission from the abdomen was the lowest: values increased along the central axis rostally to the throat. Highest values were observed over the cheeks; emission again decreased at the forehead.

The data obtained with the photomultiplier correspond with the pattern of emission from the single CCD subject described in Fig. 1, reflecting a "common" human anatomic percentage distribution emission pattern.

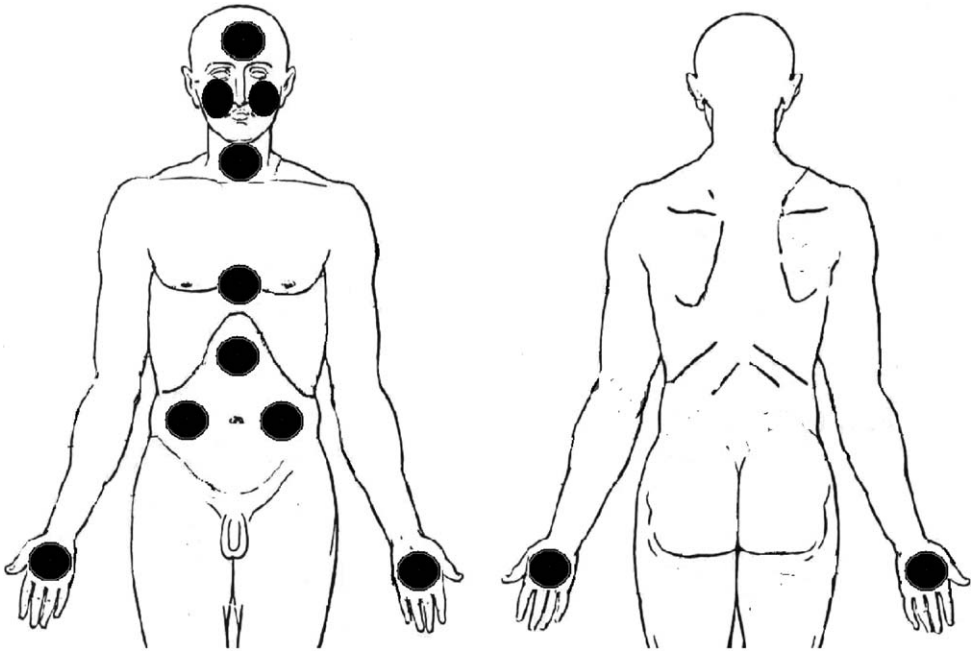

Fig. 3. Anatomic locations used to register spontaneous emission of 20 male subjects.

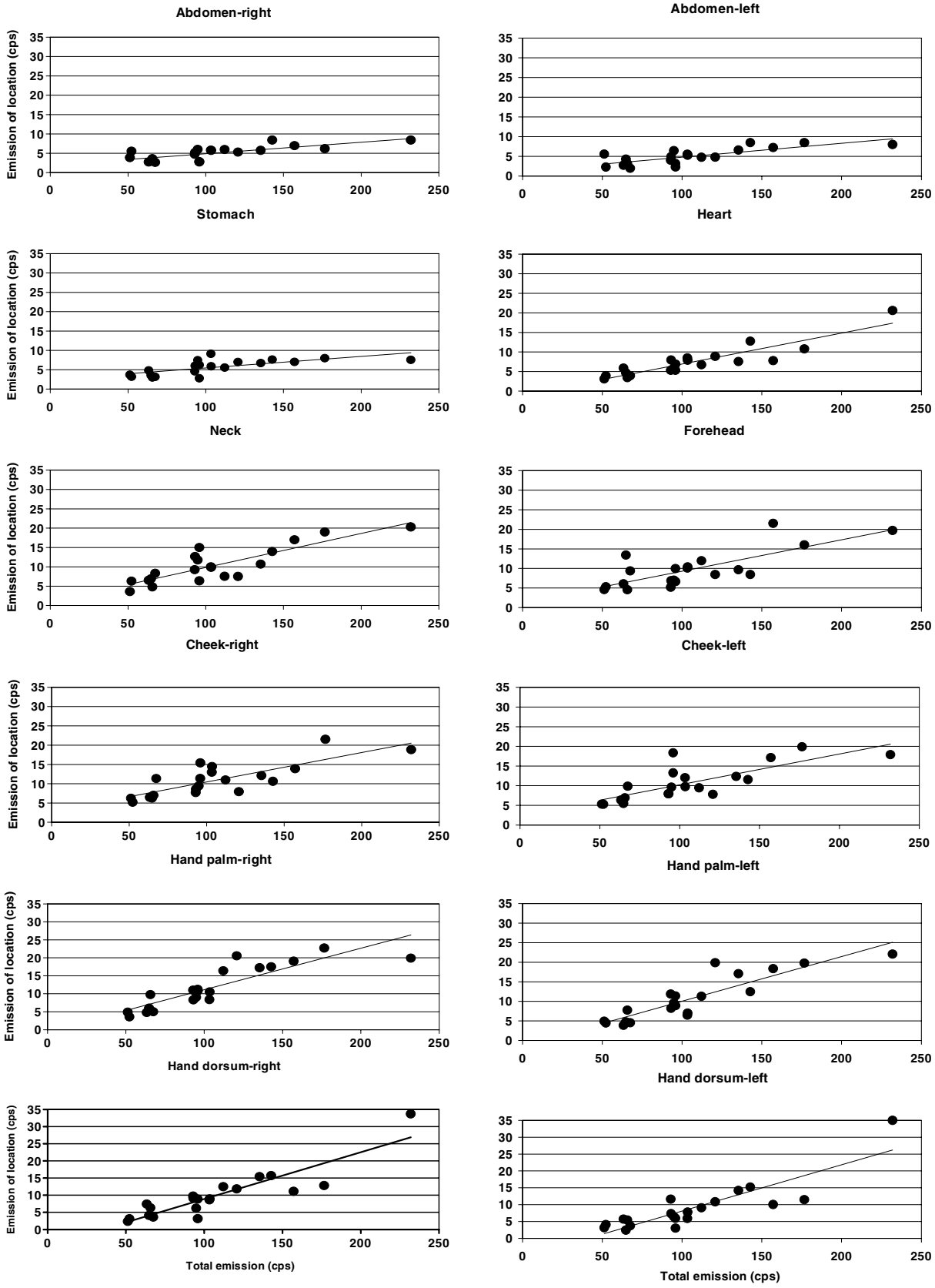

Fig. 4. Contribution of photon emission from individual anatomic locations to total emission for each subject. *X*-axis indicates total photon emission (counts/s); *Y*-axis indicates photon emission (counts/s) for each anatomic location. Each dot in a panel represents one subject, the presented line represents the best fit.

Table 2
Percentage contributions of photon emission from individual anatomic locations to total emission, and correlation of individual anatomic locations tot total emission

Anatomic location	Contribution (%)	Correlation coefficient
Abdomen-right	4.8	0.76 ($p < 0.05$)
Abdomen-left	4.7	0.78 ($p < 0.05$)
Stomach	5.3	0.70 ($p < 0.05$)
Heart	7.0	0.90 ($p < 0.05$)
Neck	9.8	0.85 ($p < 0.05$)
Cheek-right	10.3	0.80 ($p < 0.05$)
Cheek-left	10.1	0.78 ($p < 0.05$)
Forehead	9.2	0.77 ($p < 0.05$)
Hand palm-right	11.2	0.87 ($p < 0.05$)
Hand palm-left	10.1	0.89 ($p < 0.05$)
Hand dorsum-right	9.2	0.89 ($p < 0.05$)
Hand dorsum-left	8.4	0.87 ($p < 0.05$)

Data are derived from Fig. 4.

3.3. Deviations from the "common" human anatomic emission pattern

Even though data indicate that usually each anatomic location contributes in a proportional manner to the total emission, it also suggests that superimposed on the "common" human emission pattern, deviations are sometimes recorded for individual subjects. These deviations were analyzed to better understand how individual subjects as well as individual anatomic locations differ from the "common" pattern.

The analysis was completed in two steps: (a) the predicted photon emission was calculated for each subject and each anatomic location based on the total value and the percentage contributions from the different anatomic locations; (b) for each anatomic location and for each subject, the deviation from the mean for each subject was calculated (independent of its sign) and expressed as a percentage of the mean contribution of that anatomic location. The distribution of the deviations demonstrated that larger deviations seemed to occur with lower frequencies; only a few exceptionally large deviations were observed (Fig. 5).

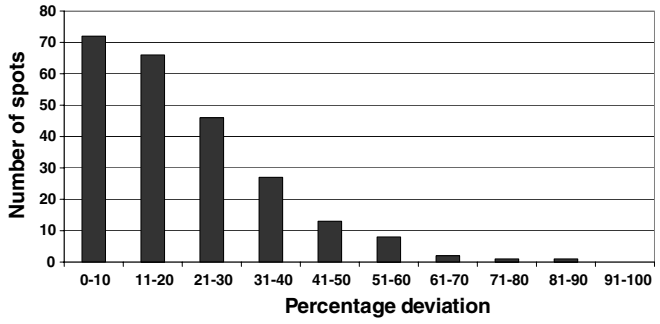

Fig. 5. Distribution of deviations from "common" pattern for all measured spots ($n = 240$). Percentage deviation was calculated according to the text.

Table 3
Average deviations of different anatomic locations from "common" anatomic percentage distribution pattern of photon emission

Anatomic location	Average deviations (%) from "common" pattern
Abdomen-right	25.3 ± 5.9
Abdomen-left	23.8 ± 6.5
Stomach	21.5 ± 4.1
Heart	16.9 ± 2.0
Neck	19.9 ± 3.5
Cheek-right	19.6 ± 3.8
Cheek-left	18.7 ± 4.6
Forehead	25.3 ± 6.2
Hand palm-right	20.3 ± 3.0
Hand palm-left	20.4 ± 3.5
Hand dorsum-right	24.7 ± 4.0
Hand dorsum-left	26.4 ± 4.7

The average deviation for each specific anatomic location is presented in Table 3. Data demonstrated deviations between 16.5% and 25.4%. The area around the heart exhibited the lowest fluctuations indicating that the contribution of emissions of that anatomic location to total emission is similar for all subjects. Deviations differ more over the abdomen, head and hand.

The average deviation for individual subjects is presented in Table 4. Data indicated that these average deviations range between 12.5% and 40.5%. Eighty-five percent of the subjects demonstrated an average deviation not exceeding 30% of the values expected on the basis of a "common" pattern.

4. Discussion

This study provides evidence that humans have a "common" pattern of ultra-weak photon emission from the

Table 4
Average deviation of different subjects from "common" anatomic percentage distribution pattern of photon emission

Subject	Average subject's deviation (%) from "common" pattern
1	20.7 ± 4.6
2	16.1 ± 2.2
3	17.6 ± 4.6
4	17.0 ± 2.6
5	28.2 ± 10.0
6	23.8 ± 9.5
7	24.9 ± 5.7
8	21.4 ± 3.4
9	14.0 ± 3.2
10	12.7 ± 3.1
11	22.1 ± 5.0
12	19.3 ± 3.2
13	14.0 ± 2.9
14	17.7 ± 4.1
15	32.0 ± 10.3
16	34.4 ± 4.3
17	28.9 ± 6.1
18	17.3 ± 3.6
19	13.9 ± 2.2
20	42.0 ± 7.9

superior anatomic parts of the body including the upper extremities. The abdomen emits the lowest intensity, which gradually increases rostally and is the highest around the face. High emission values were also documented for the hand. The pattern corresponds with photon emission images utilizing the highly sensitive technology of the CCD42 family of CCD sensors. This sensor, in combination with back thinning and extremely low noise amplifiers, allows the imaging of large area human photon emission with high resolution. The contrast of the large body images is determined by the spatial shot noise of the dark current. Signal noise ratio can be increased by expansion of the exposure time or reduction of the spatial resolution. The present images were obtained according to the technical specifications in the section on methods without further reduction of the spatial resolution.

Neither the "pattern" of emission nor the differences between subjects reflect delayed luminescence after exposure to light prior to recording. Such is excluded by sufficient adaptation to dark room conditions prior to measurements [8,9]. The emission pattern is also not explained by reflection of light from "high-emission" anatomical regions of the body, because emission intensity is too low.

The emission pattern in CCD images was recorded, utilizing a spectral sensitivity in the 400–900 nm range. The spectral sensitivity of the moveable photomultiplier was limited to the range of 200–650 nm. Research with the moveable photomultiplier has demonstrated that human photon emission was at wavelength more than 430 nm, independent of the body location [8,9]. Future studies need to quantify the specific contribution of the different parts of the spectrum to emission of different anatomical locations of different subjects.

The etiology of the "common" pattern of emission is presently unknown. The abdomen housing predominantly soft tissues presents the lowest emission, whereas the head and hands containing highly structured bone exhibit the highest emission. Further research is required to clarify relationships between emission and anatomic areas. Might this suggest a lack of electrical field homogeneity over the body surface?

Subjects demonstrated a pattern of emission that closely resembles a "common" pattern with only minor deviations over specific anatomic locations. In contrast, a few subjects exhibited larger deviations. The average deviation of individual subjects from the "common" pattern ranged between 12.5% and 40.5%. The dynamic character of the deviations is currently under investigation. The initial pilot data acquired with four subjects demonstrated that emission values of individual subjects could fluctuate during the daytime. Photon counts over the body were lower in the morning than in the afternoon with the thorax–abdomen region emitting the least and most constant emission. However, the upper extremities and the head region emitted the highest levels which increased during the daytime. It was evident that total emissions varied, whereas the "common" pattern remained almost similar during the day. Further research is required to detect if specific anatomic regions are physiologically regulated in different ways. Such research might lead to new insights regarding differential physiologic/metabolic processes involved in ultra-weak photon emission.

5. Abbreviations

CCD	charge-coupled device
cps	counts per seconds

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