



Increment of spontaneous human biophoton emission caused by anger emotional states. Proof of concept

Félix Zapata^{a,b}, Victoria Pastor-Ruiz^a, Fernando Ortega-Ojeda^{c,d}, Gemma Montalvo^{a,d}, Carmen García-Ruiz^{a,d,*}

^a Department of Analytical Chemistry, Physical Chemistry and Chemical Engineering, University of Alcalá, Ctra. Madrid-Barcelona km 33.6, 28871 Alcalá de Henares (Madrid), Spain

^b Department of Analytical Chemistry, University of Murcia, Campus de Espinardo, 30100 Murcia, Spain

^c Department of Physics and Mathematics, University of Alcalá, Ctra. Madrid-Barcelona km 33.6, 28871 Alcalá de Henares (Madrid), Spain

^d University Institute of Research in Police Sciences (UIICP), University of Alcalá, 28801 Alcalá de Henares (Madrid), Spain

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ABSTRACT

Humans spontaneously emit visible radiation from their skin, which is known as biophoton or ultra-weak photon emission (UPE). Up to date, UPE intensity changes according to different external and internal factors have been reported. However, the influence in the human UPE of negative emotional states has never been studied despite their clinic and forensic interest. In this work, an *ad hoc* system based on a high-sensitive CCD detector was developed to detect and image UPE from human's upper body. Then, the UPE emitted by the upper part of the body of nine volunteers was imaged in two different personal states: (i) when subjects were feeling relaxed; and (ii) when they were emotionally stimulated to feel anger. For all the volunteers, the relative measurement of UPE intensity was higher when feeling anger than when feeling relaxed. Due to the relevance of this disruptive idea and the horizon this knowledge can provide, this proof of concept must be followed by further experiments. An increase in the number of subjects will be accomplished in order to enhance these preliminary results and propose UPE as a new physiological parameter of negative emotional states.

1. Introduction

Biophoton emission or ultra-weak photon emission (UPE) is the spontaneous emission generated by all living systems without the need of an external excitation. It has been differentiated from the black-body or thermal radiation (also emitted by living systems because of some accumulated temperature) because the black-body radiation of living systems, including humans, lays on the infrared region [1]. On the contrary, UPE occurs within the visible region of the electromagnetic spectrum and has been mainly attributed to the relaxation of electronically excited reactive oxygen species (ROS) formed in biological systems during oxidative processes as lipid peroxidation and protein and nucleic acid oxidation [2]. UPE is not visually detectable since it is 3–6 orders of magnitude lower than the human eye visual threshold at photopic levels. This fact could be the reason why UPE received little attention until the 60's, when highly sensitive photomultipliers started to be developed and drove the expansion of this research over the world.

A step forward was made 30 years ago, when research focused on the development of UPE systems for humans. Highly sensitive photomultipliers or charge-coupled device (CCD) detectors have been used for human UPE detection. Depending on the instrumentation, different UPE parameters can be measured. The intensity of UPE (*i.e.* total photon counts, or photon counts per second) was the parameter most studied in the past because it is the one that requires the simplest instrumentation. Nowadays, the improvement in the development of CCD cameras opens the possibilities to other information as UPE imaging as 2D images. In this case, UPE intensity is registered and visualized as an image which is spatially distributed throughout the total number of pixels. This is very useful to detect UPE differences along the surface of a living being [3].

During the last 20 years, the literature has reported that UPE intensity depends on external factors (from the environment, such as the diurnal rhythm, the oxygen concentration or the exposition to external light including UV radiation) and internal factors characteristic of the body and its internal mechanism [3]. Among internal factors, the

* Corresponding author at: Department of Analytical Chemistry, Physical Chemistry and Chemical Engineering, University of Alcalá, Ctra. Madrid-Barcelona km 33.6, 28871 Alcalá de Henares (Madrid), Spain.

E-mail address: carmen.gruiz@uah.es (C. García-Ruiz).

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Table 1
Anonymized and age-ordered data of the participants.

Individual	Gender	Age range
1	Woman	18–25
2	Woman	36–45
3	Woman	46–55
4	Woman	46–55
5	Man	18–25
6	Man	18–25
7	Man	26–35
8	Man	36–45
9	Man	46–55

influence of the sex/age [4–6], the part of the body analysed [7,8], the suffering from certain diseases [9–12], the practise of meditation techniques [13–16] or the intention of imagining light [17–19] on the UPE emission has been studied. Particularly interesting are the works of Wijk et al. regarding the study of the meditation influence on UPE [13–16]. They found that the UPE intensity from the volunteers who practised transcendental meditation was lower than that of volunteers who neither practised meditation nor any other relaxation technique. According to these authors, this agrees with the fact that the stress is connected to an increased production of ROS, which could explain the lower UPE intensity in the subjects who practised relaxing transcendental meditation [14–16]. However, although the negative and fake emotions suppose internal personal states of great clinical and forensic interest, those have never been related to UPE spectral variations.

We hypothesized that the UPE spectra of an individual should change when stimulating a negative emotional state, like anger, in comparison with a relaxed state. To obtain spectral profiles, in our research group we recently tested an electron multiplying CCD (EMCCD) camera with a liquid crystal tuneable filter (LCTF) as transmission monochromator [20]. We achieved good UPE signals using an easy set-up based on a dark box for inserting the subjects hand in front of the LCTF-EMCCD camera working in the full vertical binning mode as spectra detector [20]. We acquired dark signals by registering the UPE intensity at different selected wavelengths (400, 450, 500, 550, 600, 650, and 700 nm) during a period of 10 min each. Afterwards, and considering the same wavelengths and time periods, we recorded the UPE from the left hand of the subject. Then, we filtered the spurious signals and subtracted the dark signal from the subject's hand signal. We obtained a stepped spectral pattern in agreement with previous reports showing a maximum intensity at about 600 nm, where we calculated approximately 200 photons. Although this simple set-up showed enough potential to face the UPE spectral measurements, it required a long measuring time not advisable to measure emotional states. For this reason, in this work we pursued to test a new CCD sensor and design an *ad hoc* setup to open the possibility of imaging the UPE of the upper part of individuals in order to measure their UPE images under relaxed and anger stimulated states.

2. Materials and methods

2.1. Subjects

Nine volunteers participated in the UPE measurements after being duly informed and after giving their written informed consent. Their study is part of the Explora project (CTQ201791358EXP) that has been approved by the Ethics Committee of Research and Animal Experimentation of the University of Alcalá (CEI-EA) (reference number CEI/HU/2019/24). This is a Committee accredited by the Community of Madrid to carry out the independent review of research projects as well as their monitoring during the project's runtime.

The volunteers were five males and four females from 18 to 55 years old. They did not practice any relaxation technique in their daily life. Table 1 shows the main characteristics of the participants selected by means of an anonymized questionnaire also approved by the ethical

committee.

2.2. Instrumentation

The experimental and device set-up was designed *ad hoc* for measuring UPE images of the upper part of the body, covering from the lower part of the chest (about the navel) upwards. This design comprised one sampler (sofa) for comfortably allocating the person in front of the UPE measuring system. This included a fixed aperture Sigma f/2.8 28 mm objective (Sigma Corporation, Kanagawa, Japan) connected to the entrance of a spectrograph (Kymera 193i-A, Andor, Belfast, UK) connected to an almost zero dark-noise astronomy high-sensitivity CCD camera (iKON M-934, maximum quantum efficiency in the 400–800 nm range, Andor, Belfast, UK). This UPE system and each volunteer were located inside a covering container to isolate them from any possible light source. The covering container, in turn, was inside a light-tight air-conditioned dark room for keeping the measuring system and subject under light isolated conditions and providing privacy to the subject. The dark room's window and its blinds were closed at all times whilst the door was only open for letting the subjects in or out of the measuring facilities. In addition, the door was covered by an excess of black opaque fabric which served for blocking any light passing through the door hinges, twists, corners, and angles. Likewise, the entire window area was covered with an excess of dark opaque-and-thick plastic which was fixed in place along the edges with long stripes of hard all-duty black adhesive tape. Moreover, the sides and back of the sofa as well as the backrest of the sofa were covered with black plastics. Hence, any possible reflections from the room would not be able to cross all barriers in and out of the container towards the inside where the subject was located. The computer and other devices for the control and data acquisition were situated in the next room to avoid their light emission whilst keeping personal distances. When necessary, and especially when starting and finishing the UPE acquisition, the researchers provided the volunteer with the proper instructions from the aside controlling room.

2.3. Measurement procedure

In order to know the dark counts (dark intensity) and its long-time stability, a dark-blank measurement was registered before and after analysing the subjects, *i.e.*, at least two background measurements for every measuring day. These blanks were recorded setting the same room conditions and experimental parameters as those used for the subjects measurements. They were as follows: detector/camera temperature (−90 °C), dark room temperature (20 °C), acquisition mode (single scan), acquisition time (30 min), camera focus, grating groove density (299.982 lines/mm), grating blaze (500 nm), input side slit (2500 μm), exposure time (1800 s), pixel readout rate (5 MHz; this read out rate was the slowest possible to introduce the least noise), vertical shift speed (11.29 μs), pre-amplifier gain (1×), and horizontal and vertical binning (16). The UPE images were acquired between 10:00 and 13:00 for all volunteers to reduce the UPE variability due to the diurnal rhythm. In addition, the volunteers wore a jacket and waited in a very dimly lit room for 15 min before the UPE measurement to minimize the light exposure. This was done to avoid the delayed light emission (DLE) known to be emitted by the skin during some time after its exposure to light and corresponding to the induced UPE instead of the spontaneous UPE, which was our goal in this study. Each volunteer was then guided to the dark room, where she/he managed to sit down on the comfortable single couch located in front of the CCD camera. Once seated, the volunteer undressed from the waist up. The spontaneous UPE of the volunteer's upper body (including face and torso) was then measured under the conditions indicated above.

2.4. Stimulation of the emotional/relaxed personal states

Each volunteer was measured in the same session under relaxed and

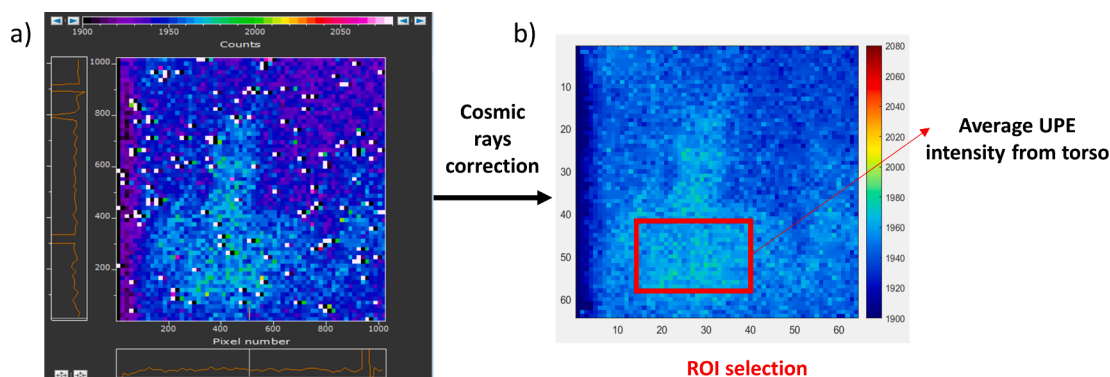


Fig. 1. Raw (a) vs. cosmic ray corrected (b) false colour images, and ROI selection (b).

anger states because this was conceived as a relative measurement. For being in a relaxed state, each volunteer was asked to be calm and relaxed whilst breathing in and out, deeply and slowly, imagining nice places where she/he would like to be or simply paying attention to the inhalations or exhalations. For measuring the anger self-stimulation states, each volunteer was asked to be angry by frowning and making angry faces while thinking about personal very upsetting things or events. The researchers helped triggering and maintaining the volunteers in the anger states by making annoying knocks and noises during the UPE measurements. These noises were provoked by friction of a metallic box (about $36 \times 20 \times 14 \text{ cm}^3$) in the outside part of the wall and the door of the room where the subject was measured. After measurements, subjects were asked to know if they achieved the pursued personal state. It was noted that all subjects were irritated and different levels of anger states were achieved according to their personal perceptions.

2.5. Data analysis

The raw images were opened and visualized within the SOLIS Software (Andor, Belfast, UK). The raw images were affected by cosmic rays as displayed in Fig. 1, that is, a considerable number of pixels had abnormal saturated intensity values. Because of that, the raw images were treated using an *ad hoc* homemade algorithm developed in Fortran language with the aim of removing the cosmic rays. Concretely, those pixels affected by cosmic rays were detected and their intensity values were replaced by the average of the 24 surrounding pixels. This way, the

result is an image that presents a continuous gradation without the highly contrasting pixels generated by the cosmic rays (Fig. 1). After the cosmic ray correction, the images were imported to the MatLab software (The MathWorks, Natick, Massachusetts, USA) where they were equally scaled and compared to each other. In addition, a region of interest (ROI) of each image was selected by using a rectangular shape involving the upper body (torso) of the person (Fig. 1). This selection was made in order to straightforwardly avoid the non-silhouettes regions, thus improving the quantitative determination of the UPE from each person. The UPE intensity average and standard deviation for the ROI pixels were then calculated. The average UPE intensity values for the relaxed vs angry subjects' states were compared together with the background's intensity. The average intensity of the background was calculated from a blank image collected every day just before each subject.

3. Results and discussion

3.1. Developing of an *ad hoc* UPE measurement system

Studying human emotional states and responses can be paramount in many medicine and psychology disciplines. However, many methods for studying such emotional states and responses are rather invasive, time consuming and their precision may not be guaranteed. Although measuring UPE is not new, in this study we explored, by the first time, the usefulness of UPE for measuring human emotional states. However, most cameras and light detecting systems do not have the resolution,

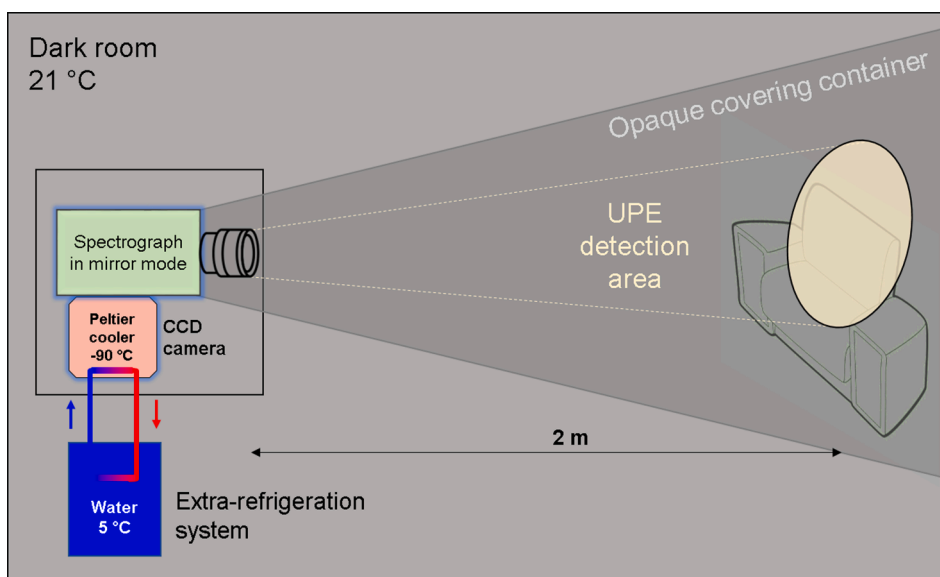


Fig. 2. Basic scheme of the *ad hoc* UPE measuring system.

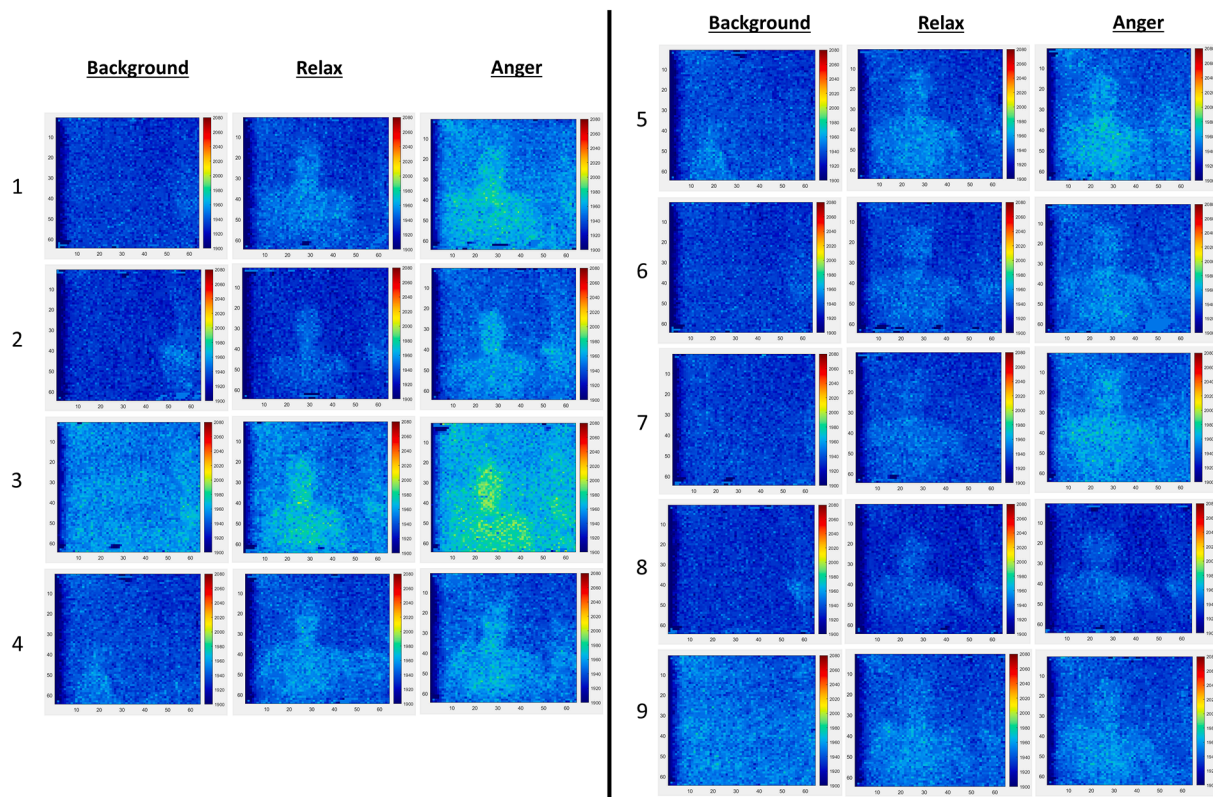


Fig. 3. UPE images from the nine participants, when they were under relaxed (middle column) or anger (right column) stimulated personal states, in comparison to their corresponding background image (left column).

sensitivity and room conditions required for trying to detect UPE in humans experiencing different emotional states. In consequence, there was the necessity of developing a new full non-invasive system that can be used for measuring UPE in those subjects.

Hence, Fig. 2 shows the scheme of the modular instrumental device developed in our laboratory to measure the human's UPE as images and spectra. This study was based on an objective and a CCD camera, since the intercalated spectrograph was only used in mirror mode to lead the light to the camera. Although the CCD camera had its own Peltier cooling system, an additional water loop refrigeration system was connected. This way, the entire working temperature of the camera did not exceed $-90\text{ }^{\circ}\text{C}$. Fig. 2 also shows the distribution of the UPE measuring system within the dark room with respect to the sofa in which the person was sitting during the UPE measurement. In brief, the distance between the objective and the sofa's backrest was around 2 m. Thus, the objective was appropriately focused to such distance. In order to further prevent any external photon from reaching the objective, the whole path from the objective to the sofa was inside an opaque container. Besides, as mentioned before, in order to stop any outside light permeations and possible emissions from the sofa and wall materials, the sofa and its surroundings (the wall behind the sofa and the opaque container) were internally covered with black opaque plastic.

Regarding the measurement conditions, it is important to remark that measuring a single scan for a long period (≥ 30 min) and using the camera's on-chip binning mode were decisive to obtain an image in which the person's silhouette was evident. Although, the on-chip binning pixelated the image, its quality was still acceptable whilst the signal-to-noise-ratio (SNR) was largely improved without adding too much readout noise. The higher the spatial binning factor, up to the 16×16 level, the more evident the person's UPE silhouette was. That is, despite losing the spatial resolution (*i.e.*, increased pixelation) because of the combination/binning of pixels, the contrast between the person's silhouette and the background was increased. As consequence, using a

16×16 binning mode during 30 min were the selected conditions to research the UPE changes under relaxation and anger stimulated states.

3.2. UPE changes under relaxation and anger stimulated states

After the initial development of the UPE measuring system for this research, the different volunteers were measured under the above-mentioned conditions. Fig. 3 shows the UPE images from the nine participants when they were feeling either relaxed or angry. When comparing the background and UPE false colour images from a person's relaxed and angry states (Fig. 3), the silhouette/outline of the person is clearly observed in the last two image columns. However, no outline is observed in her/his corresponding dark-blank background image. Unfortunately, the intensity of the dark-blank background image differed from person to person. Such a quantitative difference in the background intensity was confirmed to be due to the temperature of the detector (instrumental noise). Even though it was between -95 and $-90\text{ }^{\circ}\text{C}$ for all measurements, the background intensity differences were obtained depending on whether it was closer to -95 or $-90\text{ }^{\circ}\text{C}$.

Besides the instrumental noise due to the detector temperature, the surrounding intensity of most participants was not equally distributed due to a strange anomaly that appeared at the right hand side surrounding of the person. This unexpected anomaly is under investigation in order to be eliminated. Nevertheless, this anomaly is a systematic error that affects both the relax-measurement and the anger-measurement. Thus, it does not prevent from making the relative comparison between the relax- and anger- measurements. To this aim, when comparing the relaxed and angry UPE images at the same scale (Fig. 3), the person's silhouette/outline showed higher UPE intensity values when the subject was under the negative emotional state (anger). Interestingly, it should also be noted that not only the person was brighter when irate than when relaxed, but also its surroundings. Far from what it might seem abnormal, this result can be explained by the

Table 2

Average UPE intensity (counts) from the torso’s ROI of the nine subjects under relaxed or anger stimulated states. The average UPE intensity from the dark background is also provided.

Individual	Background	Stimulated state	
		Relaxed	Anger
1	1942 ± 7	1950 ± 7	1970 ± 7
2	1931 ± 7	1941 ± 7	1959 ± 9
3	1957 ± 7	1969 ± 8	1982 ± 9
4	1944 ± 8	1957 ± 8	1961 ± 7
5	1936 ± 7	1945 ± 7	1954 ± 8
6	1937 ± 7	1947 ± 7	1961 ± 7
7	1934 ± 8	1940 ± 7	1942 ± 7
8	1945 ± 7	1955 ± 7	1964 ± 8
9	1952 ± 7	1954 ± 7	1957 ± 8

fact that the photons from the torso-naked person’s UPE are being partially reflected by the black opaque fabric that is covering the surrounding materials. Let’s see a clearer example, if a lamp was turned on inside the compartment, not only the area of the lamp would be brighter, but also the surroundings because the light (photons) from the lamp illuminate all the surrounding materials. The person acts like an ultra-weak intensity lamp, but using a high-sensitivity camera, those photons can be detected. Thus, even though a black opaque fabric was used to cover all the surroundings and minimize the person’s UPE reflections, there is always, in every material, a significant percentage of photons’ reflection. Therefore, if the person was emitting more UPE because of the anger state, the surroundings would also be reflecting more photons, as observed in Fig. 3 for all volunteers.

All the volunteers in this study showed an UPE image more intense when they were under self-stimulated anger states in comparison with the relaxed ones (remember every person was measured the same day but under different self-stimulation states). Besides such qualitative

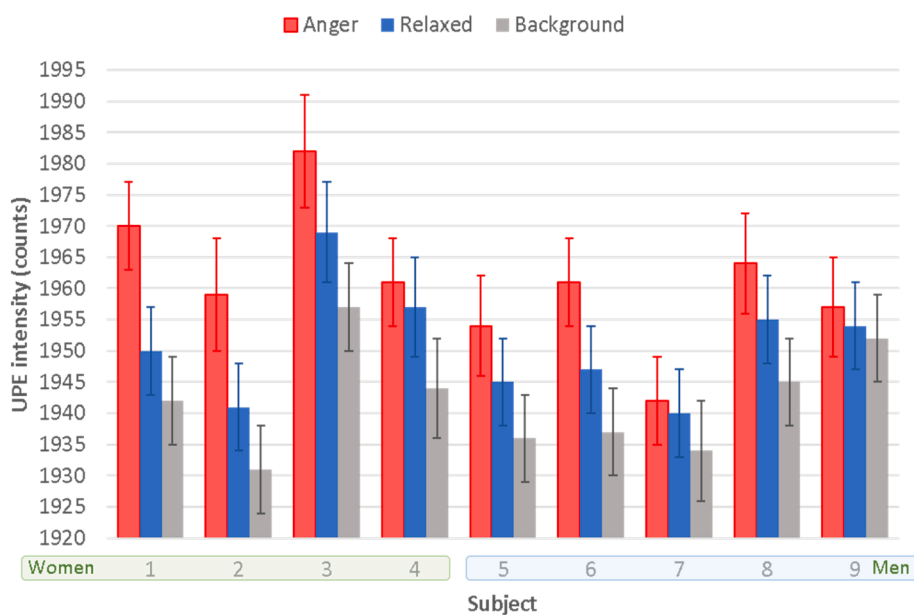
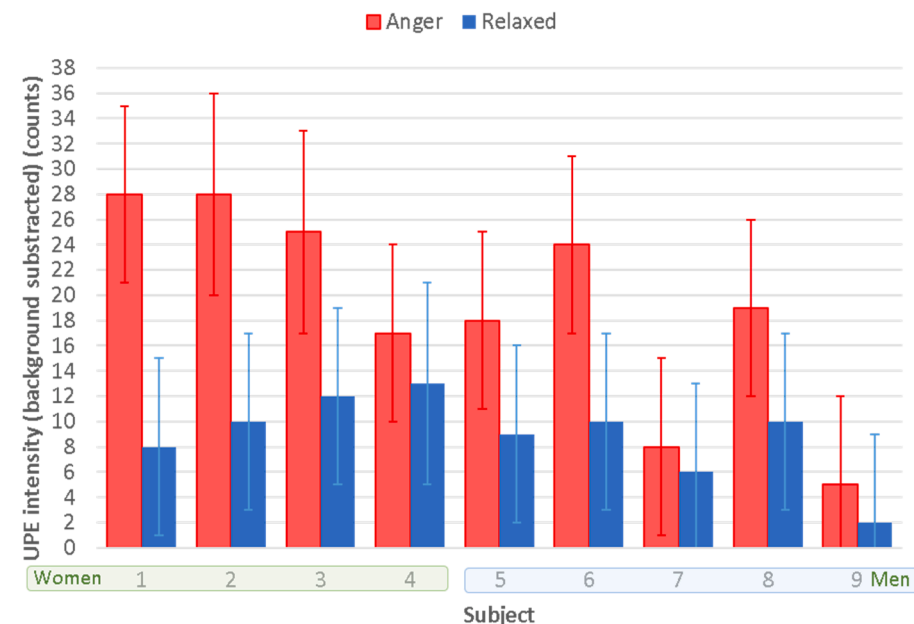


Fig. 4. UPE intensity averages from the torso of the nine subjects under relaxed or anger stimulated states. The image above shows the data before the background subtraction, whilst the image below indicates the data after the subtraction. The subjects are ordered in an ascending age fashion for the female (left) and male (right) groups, respectively. The error bars indicate the standard deviation obtained when calculating the average UPE intensity from the pixels contained within the torso (ROI) of each person.



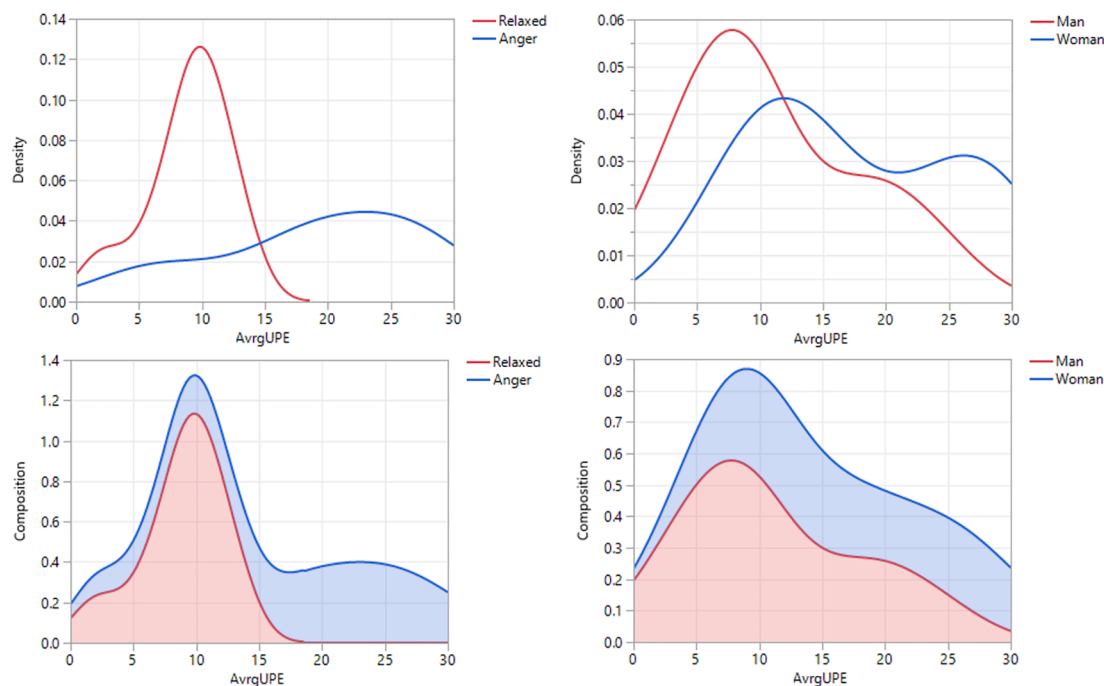


Fig. 5. Densities (top) and composition of densities (bottom) plots for the relaxed-anger (left) and women-men (right) relationships from the nine subjects under relaxed or anger stimulated states.

images comparison, the relax-anger UPE intensity differences were quantitatively determined by comparing the average UPE intensity values from each person's torso under the two different personal states (Table 2). The authors compared a same area (ROI: 25x20 pixels) from the torso of all participants instead of the whole image (surroundings included) because each person had different size. Hence, larger amounts of higher-intensity pixels were expected for bigger volunteers. Thus, the same area was taken for every person considering an area as large as possible but fitting inside every person's upper-body. This way, the average UPE intensity results of each person (Table 2) can be compared against each other without the influence of the person's size.

Anger is a negative emotional state involving dopamine/serotonin neurotransmitters release [21]. These substances probably act as internal stressors or stimuli in a chain reaction that would increase the ROS production. The ROS species would lead to the formation of high-energy intermediates, whose decomposition generates electronically excited species that undergo electronic transition to the ground state. This transition, in turn, would result in the release of photons (UPE) primarily in the VIS range, which can be measured at the skin level. Moreover, it is relevant to pay attention to the fact that the CCD sensor used in this study possesses its maximum quantum efficiency in the 400–800 nm VIS range. Therefore, the anger-related UPE increment observed in this study seems probably stimulated for the neurotransmitters generated under the negative emotional states produced during the anger-induced measurements.

Fig. 4 shows the UPE intensity averages from the torso of the nine subjects under relaxed or anger stimulated states before the background subtraction (left) and after it (right). Regardless of the subject, it is clear that the UPE intensity was higher in the subjects when they were in an anger state.

Furthermore, although the subject's sample is small, the intensity behaviour may show the presence of at least two groups of emitting people; those who emit more UPE and those emitting less of it. A means comparison (Wilcoxon/Kruskal-Wallis tests) performed on the UPE intensity data indicated that both states (relax and anger stimulated states) showed significant differences ($0.02 < 0.05$) despite that the standard deviations may lure otherwise. This seems to be the case for both the

women and men groups, being a personal factor independent of the gender.

In addition, the corresponding densities plots were calculated in order to compare the distribution and composition of the response across the levels of the State factor as displayed in Fig. 5. This figure also shows the summed densities, weighted by each group's counts, for the relaxed-anger and women-men relationships from the nine subjects under relaxed or anger stimulated states. At each UPE intensity value, the plot shows how each State group contributes to the total. As a general plain observation, the UPE intensity of the volunteers was about 0.5 to 1% (*i.e.*, around 10 counts) higher when anger was stimulated in comparison with relaxation. However, as the Fig. 4 shows, these differences were not uniform or equal along the entire UPE range. The density plots indicate that the anger self-stimulated state contributed less at the lower UPE intensities (*i.e.*, 0 to ~18), and mostly at the largest UPE intensity values (*i.e.*, ~18 to 30). This is indeed a good proof of concept that shows how different and large the UPE can be, even if the subjects were not completely angry according to their personal perception.

Regarding the Sex factor, a means comparison (Wilcoxon/Kruskal-Wallis tests) indicated that both sex (female and male) did not really show significant differences ($0.07 > 0.05$). Nevertheless, the Women-Men densities plots do show that women tested in this study emit a bit more along the largest UPE intensities values (*i.e.*, 12–30). This would be another interesting behaviour that needs additional exploration. Thus, further research with a larger subject's sample will be needed to confirm this preliminary results.

In addition, it would also be necessary to explore other methodologies to stimulate the emotional states and corroborate the UPE quantitative results. Nonetheless, it seems clear that the qualitative results are consistent because the measured UPE increased for all the subjects, even in the cases where the individuals admitted to be more upset than angry according to their personal perception.

4. Conclusions

This work shows the proof of concept that negative emotions like

self-stimulated anger increase the UPE of an individual in comparison with his/her relaxed state. These experimental results demonstrate that the UPE images from the upper part of the subjects' body were more intense in subjects under anger stimulated states with respect to the relaxed states. This fact may contribute to the perception of negative emotions even when such emotions are not declared.

In order to confirm the UPE changes in people under negative and fake emotional states, further experiments are being performed in our laboratory improving the experimental *ad hoc* measurement system and improving the methodology for stimulating emotions, complementing it with other physiological parameters like heart rate and skin conductance. Fake and negative emotional states may affect the health of the individual and also have importance in forensic investigations. As consequence, this pioneer study opens new horizons to propose UPE as a new physiological parameter able to be correlated with negative and/or fake emotions that can help in medical or forensic investigations.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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