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Abstract. Solar radiation that reaches Earth’s surface can have severe negative consequences for organisms. Both visible light and ultraviolet A (UVA) radiation are known to initiate the formation of reactive oxygen species (ROS) in human skin by photosensitization reactions (types I and II). In the present study, we investigated the role of visible light and UVA radiation in the generation of ROS on the dorsal and the palmar side of a hand. The ROS are known to oxidize biomolecules such as lipids, proteins, and nucleic acids to form electronically excited species, finally leading to ultraweak photon emission. We have employed a highly sensitive charge coupled device camera and a low-noise photomultiplier tube for detection of two-dimensional and one-dimensional ultraweak photon emission, respectively. Our experimental results show that oxidative stress is generated by the exposure of human skin to visible light and UVA radiation. The oxidative stress generated by UVA radiation is claimed to be significantly higher than that by visible light. Two-dimensional photon imaging can serve as a potential tool for monitoring the oxidative stress in the human skin induced by various stress factors irrespective of its physical or chemical nature. © 2012 Society of Photo-Optical Instrumentation Engineers (SPIE). [DOI: 10.1117/1.JBO.17.8.085004]

Keywords: two-dimensional CCD imaging; skin; ultraweak photon emission; skin chromophores; oxidative stress; UVA radiation.

1 Introduction

Various types of biotic (viral, bacterial, and fungal) and abiotic (chemical and physical) stresses are responsible for the oxidative damage in human skin. Among the physical stress factors, ultraviolet A (UVA) radiation plays a major role due to its high penetration of Earth’s surface owing to changing environmental conditions during the past decades. The exposure of the epidermal and dermal layers of human skin to UV radiation is accompanied by the formation of reactive oxygen species (ROS). To combat the deleterious action of ROS on human skin, nonenzymatic and enzymatic antioxidant defense systems have been developed by the skin. The nonenzymatic antioxidant defense system consists of low-molecular-weight components such as chromophores (carotenoids, melarins, urocanic acids, porphyrins, bilirubins, flavins, and pterins) and vitamins (A, B, C, D, and E), whereas the enzymatic antioxidant defense system is composed of various types of antioxidant enzymes (superoxide dismutase, peroxidase, catalase, and glutathione reductase). When the number of ROS exceeds a critical threshold due to the saturation in capacity of the antioxidant defense system, ROS can cause oxidative damage to the cells, leading to skin damage in forms such as premature skin aging and even skin cancer.

Under certain circumstances, the endogenous chromophores such as porphyrins (uroporphyrin, coproporphyrin, and protoporphyrin), bilirubins, melarins (eumelanin and phaeomelanin), flavins (riboflavin, flavin mononucleotide), pterins (6-carboxypterin, formylpterin, neopterin, and bioppterin), and urocanic acid (trans-urocanic acid) act as photosensitizers. A photosensitization reaction is initiated by the absorption of visible light and UVA radiation by a photosensitizer, forming the singlet excited state. The singlet excited state is known to form the triplet excited state in a photosensitizer by intersystem crossing. Among the chromophores, melarins and bilirubins, which are the predominant pigment in the human skin, have been demonstrated to absorb both in the UV and visible region of the spectrum at spectral range between 300 and 600 nm. On the other hand, other chromophores such as the urocanic acid (250 to 300 nm), riboflavins (355 nm), and pterins (345 to 375 nm) have an absorption maximum in the UV range with almost no absorption in the visible region of the spectrum. The excited photosensitizer undergoes electron transport and energy transfer, leading to the formation of radical (superoxide anion radical, O₂⁻; hydroxyl radical, HO•) and nonradical (hydrogen peroxide, H₂O₂; singlet oxygen, ¹O₂) ROS. Radical and non-radical ROS are known to oxidize lipids and proteins via hydrogen abstraction and oxygen addition, respectively.

It is well established that hydrogen abstraction from a biomolecule by HO• leads to the formation of an alkyl radical (R•), which in the presence of molecular oxygen forms a peroxyl radical (ROO•). The reactive ROO• can further extract an electron from a biomolecule forming another R• and hydroperoxide (ROOH), which is reduced by transition metals to an alkoxyl radical (RO•). Electron paramagnetic resonance study has revealed that lipid R• and RO• can be produced in skin biopsies following exposure to UV radiation. Self-reaction of two ROO• or RO• forms acyclic intermediate tetroxide, RO·•.   

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known to decompose either to triplet excited carbonyls \( [3(C = O)] \) and molecular oxygen or \( ^1O_2 \) and ground-state carbonyls \( (C = O) \). The oxidation of biomolecules mediated by \( ^1O_2 \) through oxygen addition results in the formation of cyclical intermediate dioxetane. The decomposition of dioxetane forms \( [3(C = O)] \), known to further transfer the excitation energy to molecular oxygen forming \( ^1O_2 \). The electronic transition from the excited state to the ground state of a photosensitizer is accompanied by photon emission at low intensity, which is referred to as ultraweak photon emission. It is well established that \( [3(C = O)] \) emits photons in the blue region of the spectrum, which ranges from 400 to 500 nm, while the dimol emission from \( ^1O_2 \) has been demonstrated in the red region of the spectrum at 634 nm and 703 nm.\(^{3,25,27}\)

Through oxygen addition results in the formation of \( C = O \) in the human stratum corneum. It was observed that UV-radiation-induced ultraweak photon emission in the human skin showed a maximum of photon emission in the spectral range 400 to 580 nm, supporting the assumption that \( [3(C = O)] \) is a main source of ultraweak photon emission.\(^{1,28}\) In contrast, it has previously been demonstrated that UVA-induced ultraweak photon emission is in the red region of the spectrum.\(^{30}\)

In our present study, we investigated the effect of visible light and UVA radiation on ultraweak photon emission by employing a highly sensitive charge coupled device (CCD) camera and a low-noise photomultiplier tube (PMT). The experimental results show that exposing human skin to UVA radiation enhances ultraweak photon emission, revealing oxidative stress in the human skin.

2 Materials and Methods

2.1 Subject

The following study was conducted on the author’s hand, and no other subject was involved in the study. To prevent the intervention of delayed luminescence during the measurement, the subject was dark-adapted for 30 min prior to performing measurements in a darkroom restricted from any light. To avoid any kind of diurnal fluctuation in ultraweak photon emission, the measurements were performed during a fixed period ranging from 11:00 and 14:00 h. Use of any types of cosmetics was prohibited during the course of the study. The current study was performed in agreement with the ethical principles stated in the declaration of Helsinki and its subsequent revisions.

2.2 Light Exposure

2.2.1 Visible light exposure

The dorsal and palmar sides of the hand were exposed to visible light using a Philips 60 W/240 V light source (Philips Electronics Ltd, Guildford, UK) with a spectral range of 400 to 700 nm measured by employing a LI-COR LI-1800 spectral radiometer (LI-COR Biosciences, St. John’s Innovation, Cambridge, UK).

The central area of the hand was chosen for PMT measurements, while two-dimensional (2-D) imaging of the complete surface of the dorsal and palmar sides of the hand was measured. The light source was located outside the experimental darkroom. The exposure time was 5 min, and the time between the end of irradiation and the start of measurement was kept at 20 s in each measurement. The hand was distanced 6 cm from the visible light source. The power density on the surface of the skin was 14.8 W m\(^{-2}\).

2.2.2 UVA radiation exposure

The dorsal and palmar sides of the hand were exposed to UVA radiation using a Philips UVA CLEO SWIFT lamp commonly used for tanning purposes with a spectral range of 320 to 400 nm as measured by employing a LI-COR LI-1800 spectral radiometer (LI-COR Biosciences, St. John’s Innovation, Cambridge, UK). The entire dorsal side of the hand was exposed with UVA radiation and the central area of the hand was chosen for PMT measurements, while 2-D imaging of the complete surface of the dorsal side of the hand was measured. The UVA source was located outside the experimental darkroom. The exposure time was kept at 5 min, and the hand was positioned 6 cm from the UVA source. The time between the end of irradiation and the start of measurement was kept at 20 s in each measurement. The power density on the surface of the skin was 30.6 W m\(^{-2}\).

2.3 Ultraweak Photon Emission

Ultraweak photon emission measurements were accomplished employing the CCD camera [Fig. 1(a)] and the PMT system [Fig. 1(b)] installed in a black painted inner darkroom with a

![Fig. 1 Schematic illustration of experimental setup for measurements of (a) 2-D and (b) 1-D ultraweak photon emission. The 2-D ultraweak photon emission was measured using a CCD camera, whereas 1-D ultraweak photon emission was measured using PMT.](https://www.spiedigitallibrary.org/journals/Journal-of-Biomedical-Optics)
dimension of 3 × 1.5 × 2.5 m. The measurement systems inside the inner darkroom were controlled and data were recorded with the computer located in the outer darkroom. The door in the inner darkroom was protected with a black curtain to restrict any photon entrance from the outer darkroom. For quantitative analysis, all measurements were done in three replicates.

2.3.1 Two-dimensional photon emission imaging

The highly sensitive CCD camera VersArray 1300B (Princeton instruments, Trenton, NJ, USA), with spectral sensitivity in the range 200 to 1000 nm and almost 90% quantum efficiency in the visible range of the spectrum, was employed for the 2-D photon imaging. The spectral sensitivity was limited to 350 to 1000 nm by the lenses. An objective lens of 50 mm focal distance (F mount Nikkor 50-mm, f:1.2, Nikon) was used to enhance the light collecting efficiency. The CCD unit contained a liquid-nitrogen dewar to cool the CCD element down to −110°C to reduce the dark count. The following parameters were used during the measurements: scan rate, 100 kHz; gain, 2; image format, 1340 × 1300 pixels; distance between detector and the hand, 37 cm; and accumulation time, 30 min. Data correction was made by subtracting the background signal prior to each measurement. Improvement of signal-to-noise ratio was accomplished using the binning mode with a binning factor of 4, which resulted in an image format of 335 × 325 pixels.

2.3.2 One-dimensional ultraweak photon emission

A low-noise PMT R7518P, sensitive in the spectral range 185 to 730 nm, and a photon counting unit C9744 (Hamamatsu Photonics K.K., Iwata City, Japan) were employed to measure one-dimensional (1-D) photon emission. To reduce the thermal electrons, the PMT was cooled down to −30°C using thermoelectric cooler C9143 (Hamamatsu Photonics, K.K., Iwata City, Japan). The overall noise comprising the dark count and background light was 2 counts s⁻¹. The dark count was adjusted to approximately 1.5 counts s⁻¹ at −1150 mV. To minimize the background light noise to 0.5 counts s⁻¹, PMT was kept in a vertical position. During measurements of the hand, a distance of 2 cm was kept between the hand and the PMT window.

2.4 Data Analysis

Prism 5.0 software (GraphPad Software, Inc., La Jolla, CA) was used to calculate the area under the curve expressed in thousands of counts. The mean value and standard deviations were calculated (mean ± SD, n = 3).

3 Results

3.1 Two-Dimensional Imaging of Ultraweak Photon Emission from the Dorsal Side of the Hand

Two-dimensional imaging of spontaneous, visible-light- and UVA-radiation-induced ultraweak photon emission was measured on the dorsal side of the hand using a highly sensitive CCD camera (Fig. 2). The photograph and the corresponding image of spontaneous ultraweak photon emission measured on the dorsal side of the hand are shown in the Fig. 2(a) and 2(b), respectively. To test the effect of visible light and UVA

Fig. 2 Two-dimensional imaging of the ultraweak photon emission from the dorsal side of the hand measured by a highly sensitive CCD camera. The photographs (a) and the corresponding 2-D images of spontaneous (b), visible-light-induced (c), and UVA-radiation-induced (d) ultraweak photon emission were measured on the dorsal side of the hand. In A, the photograph was obtained under weak light illumination. In B, prior to the measurements, the hand was kept in complete darkness for 30 min. In c and d, the photon emission was recorded after subsequent exposure of the hand to visible light (400 to 700 nm) and UVA radiation (320 to 400 nm) for 5 min, and the measurements were performed after a fixed interval of 20 s. Ultraweak photon emission imaging was measured with an integration time of 30 min.
radiation on ultraweak photon emission, 2-D ultraweak photon emission was monitored on the dorsal side of the hand previously exposed to visible light and UVA radiation. It is clearly evident that the photon emission from the visible-light-irradiated [Fig. 2(c)] and UVA-irradiated [Fig. 2(d)] dorsal side is higher compared to the spontaneous ultraweak photon emission [Fig. 2(b)] from the dorsal side. The photon emission from the UVA-irradiated dorsal side is higher than the visible-light-irradiated dorsal side, depicting the long-term effect of UVA radiation compared to visible light. The 2-D ultraweak photon emission imaging reveals high oxidative stress on the dorsal side of the hand upon visible light and UVA radiation compared to spontaneous ultraweak photon emission image. The observation also indicates a higher degree of oxidative stress on the dorsal side of the hand upon exposure to UVA radiation compared to visible light.

### 3.2 1-D Spontaneous Ultraweak Photon Emission from the Dorsal and the Palmar Sides of the Hand

1-D spontaneous ultraweak photon emission was measured on the dorsal and the palmar sides of the hand using low-noise PMT (Fig. 3). When the dorsal side was put below the PMT window, a count rate of 4 counts s\(^{-1}\) was observed, whereas the palmar side showed a count rate of 6 counts s\(^{-1}\). After subtraction of the dark count of the PMT, the spontaneous ultraweak photon emission from the dorsal and the palmar sides of the hand was determined to be 2 and 4 counts s\(^{-1}\), respectively [Fig. 3(a) and 3(b)]. The photon emission persists for several hours with no drop in the count rate, confirming that spontaneous ultraweak photon emission is an inherent property of the cells. The observation that the photon emission on the palmar side of the hand has a higher count rate than the dorsal side of the hand indicates that oxidative metabolic processes on the palmar side are higher than on the dorsal side of the hand.

### 3.3 Effect of Visible Light on 1-D Ultraweak Photon Emission from the Dorsal and the Palmar Sides of the Hand

To study the effect of visible light on ultraweak photon emission, the ultraweak photon emission was studied on the dorsal and the palmar sides of the hand previously exposed to visible light. When the dorsal [Fig. 4(a)] and the palmar [Fig. 4(b)] sides of the hand were exposed to visible light prior to the measurement, an increase in photon emission followed by a decrease in the steady state value was observed. The exposure of the dorsal side of the hand to visible light resulted in the enhancement to 40 counts s\(^{-1}\) and the decay in the steady state value of 5 counts s\(^{-1}\). The exposure of the palmar side of the hand to visible light brought about the increase to 120 counts s\(^{-1}\) and the decrease in the steady state value of 7 counts s\(^{-1}\). The observation that the ultraweak photon emission from the dorsal and the palmar sides of the hand exposed to visible light remains slightly higher than the spontaneous ultraweak photon emission reflects the oxidative stress generated on the dorsal and the palmar sides of the hand. The observation that the photon emission on the palmar side of the hand is higher than on the dorsal side of the hand indicates that the oxidative stress generated on the dorsal side of the hand is higher than on the palmar side of the hand.

![Fig. 3](https://www.spiedigitallibrary.org/journals/Journal-of-Biomedical-Optics on 30 Mar 2019)

**Fig. 3** One-dimensional spontaneous ultraweak photon emission from the dorsal (a) and the palmar (b) sides of the hand measured using a low-noise PMT. Prior to the measurement, the hand was kept in complete darkness for 30 min.

![Fig. 4](https://www.spiedigitallibrary.org/journals/Journal-of-Biomedical-Optics on 30 Mar 2019)

**Fig. 4** One-dimensional visible-light-induced ultraweak photon emission from the dorsal (a) and the palmar (b) sides of the hand. In A and B, 1-D ultraweak photon emission was measured after exposure of the dorsal and the palmar sides of the hand to visible light, respectively. The radiation was accomplished using a visible light source of wavelength 400 to 700 nm. The hand was exposed to the visible light just prior to measurement for 5 min, and the measurements were performed after a fixed interval of 20 s. The decay curve was measured for 30 min followed by an interruption of 20 min and further measurement of 5 min.
The palmar side is higher compared to the dorsal side upon visible light exposure.

### 3.4 Effect of UVA Radiation on 1-D Ultraweak Photon Emission from the Dorsal and the Palmar Sides of the Hand

To study the effect of UVA radiation on ultraweak photon emission, ultraweak photon emission was studied on the dorsal and the palmar sides of the hand previously exposed to UV radiation (Fig. 5). The increase and subsequent decrease in the steady state value were observed when the dorsal [Fig. 5(a)] and the palmar [Fig. 5(b)] sides of the hand were exposed to UVA radiation prior to measurement. The photon emission upon the exposure of the dorsal side of the hand to UVA radiation resulted in the enhancement to 60 counts s\(^{-1}\) and the decay in the steady state value of 8 counts s\(^{-1}\). The exposure of the palmar side of the hand to UVA radiation brought about the increase to 160 counts s\(^{-1}\) and the decrease in the steady state value of 10 counts s\(^{-1}\). The observation that the ultraweak photon emission from the dorsal and the palmar sides of the hand exposed to UVA radiation is significantly higher than the spontaneous photon emission indicates that the oxidative stress is generated on the dorsal and the palmar sides of the hand. The observation that the photon emission on the palmar side of the hand is considerably higher than on the dorsal side of the hand indicates that the oxidative stress generated on the palmar side is higher compared to the dorsal side upon UVA exposure.

![Fig. 5](image-url) One-dimensional UVA-induced ultraweak photon emission from the dorsal (a) and the palmar (b) sides of the hand. In A and B, 1-D ultraweak photon emission was measured after exposure of the dorsal and the palmar sides of the hand to UVA radiation, respectively. The irradiation was accomplished using a UVA source of wavelength 320 to 400 nm. The hand was exposed to the UVA radiation just prior to the measurement for 5 min, and the measurements were performed after a fixed interval of 20 s. The decay curve was measured for 30 min followed by an interruption of 20 min and further measurement of 5 min.

### 3.5 Efficacy of Ultraweak Photon Emission from the Dorsal and the Palmar Sides of the Hand upon Visible Light and UVA Irradiation

To quantify the effect of visible light and UVA radiation on the ultraweak photon emission, the area under the curve was calculated (Fig. 6). The area under the curve was increased by the exposure of the hand to visible light and UVA radiation. After the visible light exposure, the area under the curve from the dorsal and the palmar sides of the hand was recorded to be two and three times higher, respectively, compared to the area under the curve of spontaneous ultraweak photon emission [Fig. 6(a)]. The exposure of the dorsal and the palmar sides of the hand to UVA resulted in the area under the curve being about four times and six times higher, respectively, than the area under the curve of spontaneous ultraweak photon emission. These observations indicate that UVA radiation generates high oxidative stress on the hand compared to visible light and that both visible light and UVA radiation generate oxidative stress on the hand.

### 4 Discussion

Chromophores play a crucial role in the formation of ROS via photosensitization composed of either electron transfer (type I) or energy transfer (type II) reactions\(^{16,35}\) (Fig. 7). In the photosensitization reaction, the absorption of excitation energy by a photosensitizer causes a transition from the ground state to the singlet excited state of the photosensitizer, which is later converted to the triplet excited states via intersystem crossing. The ROS formed by both type I (\(O_2^{**}, H_2O_2, HO^*\)) and type II (\(O_2\)) photosensitization reactions have a capability to oxidize lipids and proteins (Fig. 7). As a by-product of oxidation of lipids and proteins, reactive intermediates such as dioxetane.
and tetroxide are formed, which further decompose either to \(3(C=O)\) and molecular oxygen or \(^1O_2\) and \(C=O\) (Fig. 7). The triplet excited carbonyls can emit in the broad range from 400 to 500 nm, while \(^1O_2\) can undergo dimerization resulting in the dimol emission at 634 and 703 nm. In the present study, 2-D ultraweak photon emission was employed as a non-invasive tool to monitor the visible-light- and UVA-radiation-induced oxidative stress of the hand through photosensitization reactions (Figs. 2, 4, and 5).

### 4.1 Visible-Light-Induced Oxidative Stress on the Dorsal Side of the Hand

The observation that photon emission from the visible-light-irradiated dorsal side of the hand is higher than from the unexposed dorsal side of the hand reveals that the visible light caused oxidative stress in the human skin [Figs. 2(b), 2(c), 3(a), and 4(a)]. Melanins and bilirubins are known to absorb in the wavelength range of 300 to 600 nm. It has previously been reported that the absorption of visible light by melanins and bilirubins initiates photosensitization, leading to the formation of ROS either by type I or type II reaction.\(^{40}\) Similarly to light-induced enhancement of ultraweak photon emission, it has recently been demonstrated that the topical application of ROS (\(O_2^{\ast\ast}\), \(H_2O_2\), and \(HO^\ast\)) on the dorsal side of the hand resulted in the enhancement in ultraweak photon emission.\(^{43}\)

Supporting the role of ROS in ultraweak photon emission, it was demonstrated that various ROS scavengers (ascorbate, glutathione, CoQ10, and \(\alpha\)-tocopherol) considerably suppressed the ultraweak photon emission.\(^{44}\) The damage to the healthy cells occurs by photosensitization, which proceeds as a consequence of the photodynamic therapy. It has been suggested that the use of blue-light therapy should be done under controlled conditions, thereby preventing photodamage to healthy cells.\(^{45}\)

### 4.2 UVA-Radiation-Induced Oxidative Stress on the Dorsal Side of the Hand

The finding that the photon emission observed from the UVA-irradiated dorsal side of the hand is higher compared to the unexposed dorsal side indicates that UVA radiation induced oxidative stress in human skin [Figs. 2(b), 2(d), 3(a), and 5(a)]. Besides melanins and bilirubins, which absorb in a broad range of the spectrum from UV to the visible region (300 to 600 nm), the chromophores such as urocanic acids (250 to 300 nm), porphyrin (protoporphyrins) (320 to 400 nm), flavins (345 to 375 nm), and pterins (345 to 375 nm) absorb particularly in the UV region of the spectrum.\(^{18,20}\) The absorption of UV radiation by these chromophores initiates photosensitization, which leads to the formation of ROS either by type I or type II reaction.\(^{36,37}\) As a consequence, there is a development in human skin of both photoaging, characterized by deep
wrinkles, and cancer. Our observation that UV A-radiation-induced ultraweak photon emission is higher compared to visible-light-induced ultraweak photon emission shows a higher degree of oxidative stress on the dorsal side of the hand. It is proposed here that it is due to the large amount of photosensitizers absorbing in the UV region of the spectrum. Visible light has a higher capability to penetrate to the dermis layer of the skin, whereas UV light penetrates to the epidermal layer of the skin.

4.3 Comparison of Visible-Light- and UVA-Radiation-Induced Oxidative Stress on the Dorsal and Palmar Sides of the Hand

The result that ultraweak photon emission from the palmar side of the hand is higher compared to the dorsal side of the hand upon the visible light radiation reflects the higher oxidative stress on the palmar side [Fig. 5(a) and 5(b)]. It has previously been reported that the palmar side of the hand has low melanin content than the dorsal side, whereas the content of other photosensitizers in the dorsal side is more likely to be comparable to the palmar side of the hand. The dorsal side of the hand mainly contains the black and brown pigment, the eumelanin that acts as an antioxidant, whereas the palmar side of the hand contains a low amount of eumelanin and is more prone to oxidative damage. Because the dorsal side contains a high amount of eumelanin, the elimination of ROS formed by type I and type II reactions is highly efficient, and thus the ultraweak photon emission from the dorsal side is comparatively lower than from the palmar side of the hand previously exposed to the visible [Fig. 3(a) and 3(b)] and UV [Fig. 4(a) and 4(b)] radiation. As the palmar side of the hand has low content of eumelanin, the scavenging of ROS formed by type I and type II reactions is less efficient and leads to high ultraweak photon emission from the palmar side compared to the dorsal side of the hand previously exposed to the visible [Fig. 3(a) and 3(b)] and UV [Fig. 4(a) and 4(b)] radiation. In agreement with these considerations, it has previously been claimed that the higher spontaneous ultraweak photon emission from the facial skin might be caused by high melanin content.

5 Conclusion

The detailed mechanism of ultraweak photon emission has not been clarified completely although it is adequately understood. It is proposed here that ultraweak photon emission can act as a noninvasive method for analyzing the physiological and pathological state of the human skin. The quantitative analysis provides information on the degree of oxidative stress in the human skin, including the viable epidermis and dermis. The application of ultraweak photon emission for monitoring the effect of UVA stress on human skin is highly important for the development of effective photoprotective agents in human skin against UVA radiation.

Acknowledgments

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