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# Polychromatic spectral pattern analysis of ultra-weak photon emissions from a human body

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ABSTRACT

Ultra-weak photon emission (UPE), often designated as biophoton emission, is generally observed in a wide range of living organisms, including human beings. This phenomenon is closely associated with reactive oxygen species (ROS) generated during normal metabolic processes and pathological states induced by oxidative stress. Application of UPE extracting the pathophysiological information has long been anticipated because of its potential non-invasiveness, facilitating its diagnostic use. Nevertheless, its weak intensity and UPE mechanism complexity hinder its use for practical applications. Spectroscopy is crucially important for UPE analysis. However, filter-type spectroscopy technique, used as a conventional method for UPE analysis, intrinsically limits its performance because of its monochromatic scheme. To overcome the shortcomings of conventional methods, the authors developed a polychromatic spectroscopy system for UPE spectral pattern analysis. It is based on a highly efficient lens systems and a transmission-type diffraction grating with a highly sensitive, cooled, charge-coupled-device (CCD) camera. Spectral pattern analysis of the human body was done for a fingertip using the developed system. The UPE spectrum covers the spectral range of 450–750 nm, with a dominant emission region of 570–670 nm. The primary peak is located in the 600–650 nm region. Furthermore, application of UPE source exploration was demonstrated with the chemiluminescence spectrum of melanin and coexistence with oxidized linoleic acid.



## 1. Introduction

Ultra-weak photon emission (UPE) from living organisms, often designated as biophoton emission [1], is a commonly known phenomenon observed in virtually all living organisms [2]. Generally speaking, UPE intensity is 1/1000 times weaker than human-eye sensitivity. It is a distinct phenomenon known as bioluminescence that is faint, but detectable. It is specific to species bearing the distinctive luciferin–luciferase enzymatic system. Actually, UPE does not originate in a specific reaction or substance. Rather, it is a by-product of biochemical reactions in which excited species are formed through oxidative reaction with biomolecules. It is therefore not specific to any species or group of organisms ranging from microorganisms to plants and animals. In most cases of this chemical excitation process, reactive oxygen species (ROS) are known to be involved. For instance, in ROS-triggered lipid peroxidation, triplet excited carbonyls, and fluorescent pigments excited through energy transfer from the carbonyl groups are recognized as UPE sources [3].

Human beings are no exception: UPE, which is detectable on the skin surface [4–7], can be presented as a two-dimensional image. We previously achieved imaging of spontaneous UPE from human bodies using a highly sensitive cooled charge-coupled-device (CCD) camera with a highly efficient lens system [4,5]. We reported its behavior and its relation to metabolic activity expressing a diurnal rhythm [5].

Typically, UPE phenomena are associated with ROS production in biochemical reaction processes occurring during normal metabolism and abnormal states leading to disease. Thereby, UPE intensity and its variations reflect metabolic activity, and/or state of oxidative stress, an imbalance between the amount of ROS production and antioxidant capacity. In fact, from the time of initial discovery of UPE phenomena, it has long been anticipated for its potential for non-invasive use in assessing living bodies as media carrying pathophysiological information. Nevertheless, characterization of the complexed mechanisms has remained challenging. Practical application is restricted. Spectral information is fundamentally important to ascertain photon emission sources and mechanisms of UPE. However, the weak intensity does not allow the use of commercially available spectroscopic devices that incorporate diffraction grating. Conventionally, filter-type spectroscopy systems are used for UPE analysis: a series of optical filters bearing high-pass or band-pass transmission characteristics is incorporated with a mechanical device for filter exchange. Apparently, this method based on a monochromatic scheme has low temporal resolution that is dependent on the number of filters that determine the wavelength range and

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resolution. It is available for phenomena that are temporally stable, with stable intensity and wavelength. To overcome this shortcoming of low temporal resolution, polychromatic spectroscopy includes a dispersive device and a multi-element detector such as a linear array sensor or a two-dimensional imager [8,9]. However, when applying the device for UPE spectroscopy, restriction of the sampling area (volume) must be regulated by an optical input slit size (width and length), which strongly affects detection capabilities. Markedly higher optical throughput and total efficiency of the system, in contrast to general-use polychrometer systems, are important benefits.

We have developed a polychromatic spectral analysis system specifically for use with UPE measurements, incorporating highly transparent and efficient lens systems for a collimator and condenser optics with careful design considering the substantial improvement of transmittance and the f-number. The dispersion element that we used is a transparent diffraction grating inserted between the collimator and the condenser lens. These elements include a spectroscopy unit that can be attached to a highly sensitive cooled CCD camera. The lens systems were designed to improve throughput specifications primarily at the expense of spatial resolution, corresponding to wavelength resolution. The wavelength resolution is determined by the input slit width, lens system aberrations, and the CCD camera spatial resolution. To increase the optical input, we specifically examined spectral pattern determination with consideration of its features. The UPE spectra are generally broad. Therefore, narrow-line determination of the spectrum is less important. Even at poor wavelength peak-pair resolution, polychromatic measurements are more effective at extracting spectral features than by filter-type spectroscopy because, as a result of the convolution with the instrumental function, the observed pattern consists of wavelength information beyond the peak-pair resolution.

This report describes the system developed for spectral pattern analysis of UPE with polychromatically observed spectra of human UPE on the skin surface, demonstrating the capabilities of spectral pattern analysis of UPE for its characterization.

## 2. Polychromatic Spectrum Analysis System Designed for UPE Study

Fig. 1 presents a schematic illustration of the system developed for human UPE spectrum analysis. A measurement target, a fingertip, is placed directly under a 1 mm wide × 20 mm high optical input slit. The incident light enters a specially designed collimating lens system, which improves the f-number and transmittance by reducing the number of lens elements with the cost of the aberration properties. The collimated light passes through the transmission-type diffraction grating (GT50–03; Thorlabs, Inc., USA), which has 300 lines/mm grooves with a 17.5° blaze angle. The 50 mm × 50 mm diffraction grating covers transmission wavelengths of 400–900 nm with efficiency higher than 40%, peaking at 530 nm with 75% efficiency. Diffracted light from the grating is focused onto a cooled CCD camera with a condenser lens having the same specifications as those of the collimating lens system. The CCD camera (SI 600s; Spectral Instruments Inc., USA) provides sufficient sensitivity performance for UPE imaging, which incorporates a back-illuminated-type 25 mm × 25 mm active area CCD (CCD42-40; e2v Inc., USA) with a cryogenic unit for −103 °C cooling. Spectral sensitivity of the CCD extends from the visible to the near-infrared region. The region with quantum efficiency higher than 50% is 400–900 nm. The CCD camera was operated with 8 × 8 binning mode, corresponding to the practical pixel number of 256 × 256 with size of 100 μm × 100 μm. The minimum detectable optical power within the slit area is estimated roughly as $1.0 \times 10^{-18}$ W at 600 nm in the 20 min exposure-time condition, considering the total optical efficiency of the spectroscopy unit. The peak-pair resolution of the wavelength was measured as approx. 80 nm, as determined by the slit size, reciprocal linear dispersion (120 nm/mm), binned sizes of the CCD, and the focal length of the condenser lens.

Data processing to derive the UPE spectral pattern was conducted using the following steps: (1) accumulation of CCD data along the longitudinal direction; (2) conversion from pixel number to wavelength using the wavelength calibration formula, which is determined preliminarily by measurements using laser light sources and a monochromatic light source system (SPG-120; Shimadzu Corp., Japan) incorporated with a halogen lamp; (3) subtraction of background photon emission, which mainly originates in the material of optical components; and (4) calibration of total spectral efficiency of the system, which is determined primarily by the spectral properties of CCD quantum efficiency and transmittance of the diffraction grating. Fig. 2 presents the total spectral efficiency curve measured using a calibrated optical power meter (ML9001; Anritsu Corp., Japan) with the monochromatic light source. Regarding the noise level, the spectral pattern was treated with smoothing through a moving average within wavelength resolution.

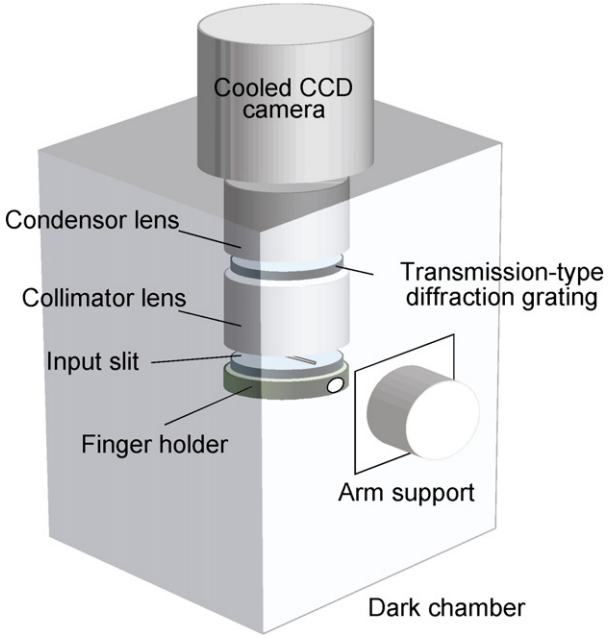


**Fig. 1.** Schematic illustration of the polychromatic spectral analysis system for human UPE.

## 3. Spectral Pattern Analysis of Human Body UPE

### 3.1. Measurement Method

The measurement region of UPE was the fingertip of the ventral side of the index finger. One author (T. I.), a healthy 21 year old man (skin

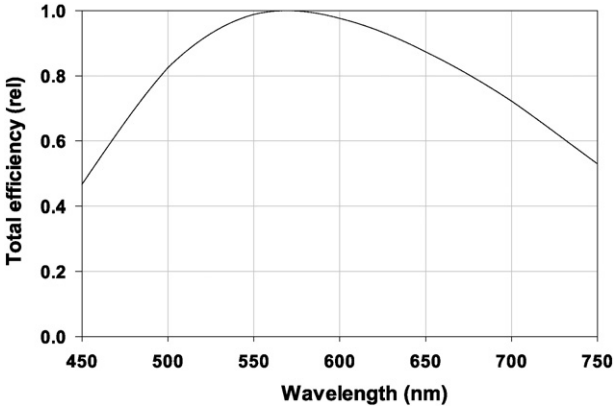


**Fig. 2.** Total spectral efficiency of the polychromatic spectral analysis system.