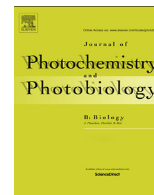
Journal of Photochemistry and Photobiology B: Biology 139 (2014) 34–38

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol



Highly sensitive imaging for ultra-weak photon emission from living organisms

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ARTICLE INFO

Article history:
Available online 1 December 2013

Keywords:
Ultra-weak photon emission
Biophoton
Imaging
CCD
Photon-counting
Reactive oxygen species

ABSTRACT

Spontaneous ultra-weak photon emissions (UPEs) are from living organisms. Often designated as biophoton emissions, they are associated with reactive oxygen species production. They have long been explored for use in the extraction of pathophysiological information of living bodies. Because of its potential non-invasiveness and because it is completely passive, it has been anticipated for application to human diagnosis. However, because of the weakness of its signal and the complexity of the mechanisms, practical applications of UPE and efforts have remained restricted.

Imaging of UPE is a powerful tool for the practical application of UPE. Furthermore, efforts to develop imaging technique have been made from the early period of UPE study. This report explains the history of UPE study, particularly describing the development of imaging technology and its application covering agriculture and medicine are reviewed. Furthermore, the issue of what was achieved and what is necessary for the additional advancement of UPE will be discussed for practical application.

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1. Introduction

Ultra-weak photon emissions (UPEs) from living organisms, often designated as biophoton emissions, occur as widely known phenomena that are widely observed in living organisms. Although photon emissions are accompanied by biological functions, their intensity is extremely weak, typically 3–6 orders lower intensity than the threshold of human visual recognition. Photon emission originates via reactive oxygen species (ROS) generated under processes of biochemical reactions with normal metabolism and/or an abnormal state promoted by ROS. Intensity variations of UPE reflect metabolic activity or changes of balance between the ROS level and antioxidant capacity. The emissions can provide valuable information about the state of oxidative stress without invasion of a monitor. Actually, UPE has long been anticipated for use as a signal mediator carrying pathophysiological information. Because of its potential for non-invasiveness and complete passivity, it has attracted attention as offering an 'ultimate' methodology for the diagnosis of human illness. Although numerous phenomena of UPE have been described during the last half-century, the practical application of UPE has remained restricted because of the weakness of the signal and the complexity of its related mechanism. To characterize and extract information, the spectral, spatial, and other optical properties including photon statistics should be used in such a single-photon level. In Japan, Professor Inaba, a world pioneer in this field, and his biophoton projects have led research in this field since the 1970s. His work has achieved the development of various technologies for precise measurement while pursuing their mechanisms.

To identify the properties of UPE for extracting valuable information, visualization as two-dimensional (2D) images is necessary for non-invasive diagnosis. This paper presents a review of the techniques and systems developed for UPE imaging. Efforts to achieve UPE imaging have been undertaken from the mid-1980s. Characteristics of each technique and evaluation of experimentally obtained results indicating the feasibility of UPE imaging for practical applications are discussed with attention to applications in fields such as medicine, agriculture, and other bio-industries.

2. UPE imaging technology

In the history of UPE research, the first imaging was accomplished using an imaging system employing a 2D photon-counting tube. After that, to the present day, the scientific grade of charge-coupled-devices (CCDs) with cooling equipment has been generally used for UPE imaging. Here, these two types of imaging devices and imaging systems are reviewed, with evaluation of a lens system, which is a key device for UPE imaging.

2.1. Two-dimensional photon-counting imaging system

The development of a 2D photon-counting tube [1] comprising a uniform photocathode, a set of micro-channel plates (MCPs), and

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http://dx.doi.org/10.1016/j.jphotobiol.2013.11.011

a position-sensitive-anode (also designated as a resistive anode or a position sensitive device) triggered the study of UPE imaging. An MCP is an electron multiplying device which conserves the spatial information of released photoelectrons from the photocathode. The position-sensitive-anode identifies an *X*–*Y* coordinate of a multiplied photoelectron-pulse from MCP through calculation. The pulses are accumulated as the number of events in the corresponding address on the frame memory in an image processor. Consequently, a photon-counting image is depicted with a gray-scale indication of the number of photoelectrons in each pixel. A schematic diagram of a typical 2D photon-counting tube is portrayed in Fig. 1.

The first image of UPE from a living organism was demonstrated using a germinating soybean, indicating intense emissions from the active area of mitosis [2]. The experiment uses the photon imaging acquisition system (PIAS; Hamamatsu Photonics KK, Japan) that has the active diameter of a photocathode measuring 15 mm with the condition of 1 h 33 min exposure time. To increase the device performance for UPE imaging, a large active area of the photocathode is beneficial. A 2D photon-counting tube with effective diameter of photocathode measuring 40 mm was developed (imaging photon detector, IPD; Photek, UK). It contributed to the success of UPE image of a rat brain *in vivo* [3].

The other type of 2D photon-counting imager system is assembled with an image-intensifier (I.I.) that has the similar configuration to that of the above-described 2D photon-counting tube. It consists of a photocathode, a set of MCPs, and a phosphor screen instead of the position-sensitive anode, in which the image on the photocathode is enhanced on the phosphor screen. The converted image on the phosphor screen is captured using a general imaging device such as a CCD camera or a conventional TV camera via a lens system or a fiber-optic plate. Single photoelectron events shown as bright spots on the phosphor screen are detected using a video-rate camera. They are recorded as counts of photoelectron events to produce a photon-counting image. The schematic configuration of a typical I.I.-based 2D photon-counting imager is presented in Fig. 2, which was assembled with an I.I., a CCD camera, and a fiber-optic plate for image coupling from the phosphor screen to the CCD camera. Camera systems of these types include full forms of the ICCD and IICCD camera.

The UPE images from a germinating soybean seed under intact conditions and injured conditions were reported using an I.I.-based 2D photon-counting imager (video intensified microscope, VIM; Hamamatsu Photonics KK) [4]. The active area of the photocathode was 12 mm diameter; 2500 s exposure time was necessary for this experiment.

The detection limit, defined as the minimum detectable photon-number, is determined by the photocathode quantum efficiency

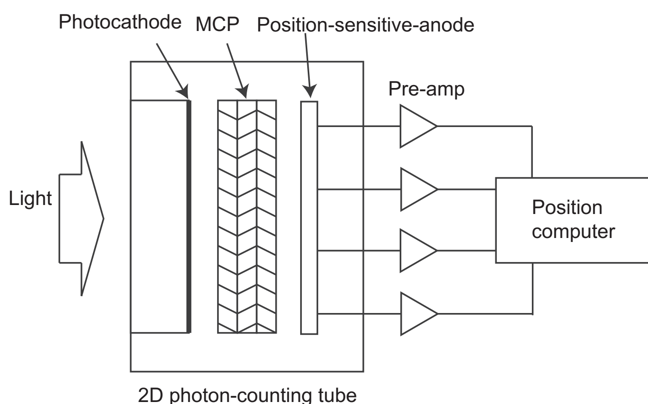

Fig. 1. Schematic diagram of a 2D photon-counting tube.

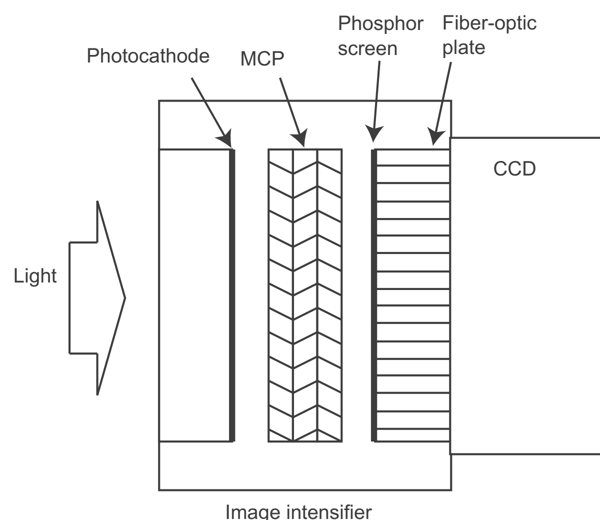

Fig. 2. Schematic diagram of an ICCD camera, as one type of I.I. based 2D photon-counting system.

and the dark current shot noise. In the case in which magnification gain of MCP is sufficient to identify single-photoelectron events, the minimum detectable photon-number is calculable from the equation of signal-to-noise ratio (SNR) represented as presented below.

$$\frac{S}{N} = \frac{\eta\langle N_P\rangle}{\sqrt{\eta\langle N_P\rangle + \langle N_d\rangle}}\sqrt{T} \quad (1)$$

Therein, $\langle N_p\rangle$ and $\langle N_d\rangle$ respectively represent the averaged photon-number and averaged dark counts per pixel in unit time. Here, η and T respectively denote the photocathode quantum efficiency and exposure time for imaging. The minimum detectable photon-number $\langle N_p\rangle_{min}$ is definable under the condition of $S/N = 1$ with $\langle N_p\rangle \ll \langle N_d\rangle$. It is derived as shown below.

$$\langle N_p\rangle_{min} = \frac{1}{\eta}\sqrt{\frac{\langle N_d\rangle}{T}} \quad (2)$$

The 2D photon-counting system provides the benefit of identifying single-photoelectron events in real time, but its spatial resolution is restricted by the performance of the MCP and the position-sensitive-anode, or the resolution of the phosphor screen in the case of an I.I.-based imaging system. The spatial resolution is generally worse than that of the cooled-CCD camera system, as described in the next section.

2.2. Cooled-CCD camera system

CCDs for scientific measurement, which were developed primarily for astronomy, have extremely high sensitivity for ultra-weak light detection. These are also suitable for UPE imaging in the field of biology. The first imaging of UPE of living organisms using a CCD camera (Photometrics, Inc., USA, with Tektronix Inc., CCD) was reported using germinating soybean seedlings [5]. The back-illuminated type, shown as Fig. 3, has excellent sensitivity. Its quantum efficiency at the peak wavelength is close to 90%, even in a cooling condition. Although the CCD has no magnification gain itself, it has better performance for weak light detection than the 2D photon-counting tube described above. That performance is achieved through extension of the integration time and reduction of the amplifier noise (designated as readout noise). The higher quantum efficiency leads to its superiority in terms of performance. For UPE imaging, a large active area for efficient light

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