Materials Today Bio 28 (2024) 101238

Contents lists available at ScienceDirect

Materials Today Bio

journal homepage: www.journals.elsevier.com/materials-today-bio

Accumulated in-situ spectral information analysis of room-temperature phosphorescence with time-gated bioimaging

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ARTICLE INFO

Keywords:
Room-temperature phosphorescence (RTP)
Time-gated microscopy
In-situ spectroscopy
Autofluorescence
Signal accumulation

ABSTRACT

This study introduces the time-gated analysis of room-temperature phosphorescence (RTP) for the in-situ analysis of the visible and spectral information of photons. Time-gated analysis is performed using a microscopic system consisting of a spectrometer, which is advantageous for in-situ analysis since it facilitates the real-time measurement of luminescence signal changes. An RTP material hybridized with a DNA aptamer that targets a specific protein enhances the intensity and lifetime of phosphorescence after selective recognition with the target protein. In addition, time-gated analysis allows for the millisecond-scale imaging of phosphorescence signals, excluding autofluorescence, and improves the signal-to-background ratio (SBR) through the accumulation of signals. While collecting the time-gated images and spectra of RTP and autofluorescent materials simultaneously, we develop a method for obtaining phosphorescence signals by means of selective exclusion of autofluorescence signals in simulated or real cell conditions. It is confirmed that the accumulated time-gated analysis can provide ample information about luminescence signals for bioimaging and biosensing applications.

1. Introduction

In the microscopic analysis of living organisms such as cells and tissues [1], autofluorescence is a drawback for overlapping photoluminescence (PL) signals [2,3]. To overcome this drawback, methods have been developed to avoid the autofluorescence of organisms, such as lifetime mapping and time-gated imaging [4–6]. Lifetime mapping typically uses time-correlated single-photon counting (TCSPC) for measurements, where fluorescence lifetime imaging microscopy [7–9] is used for fluorescent materials and phosphorescence lifetime imaging microscopy (PLIM) [10,11] for phosphorescent materials. In the case of multiple luminescence signals, a clearly distinguished mapping image can be obtained by visualizing the lifetime value of each luminescence signal [12]. However, there is a limitation that the spectral information of each signal cannot be confirmed. In addition, PLIM should be repeated to perform a new triplet population after the decay is completed and scan all x and y pixels in a detection area. This results in a long measurement time and a high cost of computational equipment [13]. Time-gated imaging is based on the combination of an optical chopper and materials with relatively longer lifetimes compared to fluorescence [14–16]. This method can be used to precisely control the delay time to submicroseconds using an electric signal [17,18]. A time delay exists between the excitation of luminescent materials and the detection of signals. Hence, only long-lived luminescence signals are selectively detected after autofluorescence disappears [19]. In general, signal detection becomes easier as the lifetime of a luminescent material increases [20]. For in vivo bioimaging applications, a luminescent material with a long lifetime is injected into a mouse, and the afterglow of luminescence is measured through time-gated analysis [21,22]. From a microscopic perspective, only the number of detected photons is important for visualization, even though the luminescent signal contains more information.

In this study, we have developed a time-gated microscopic system by adding a spectrometer to analyze the spectral information of photons. It is possible to simultaneously obtain the visible information from the microscope and spectral information from the spectrometer. The emission spectrum is essential for the quantitative analysis of luminescence across a broad wavelength range, and it involves parameters such as the

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https://doi.org/10.1016/j.mtbio.2024.101238
Received 27 June 2024; Received in revised form 10 August 2024; Accepted 10 September 2024
Available online 14 September 2024
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