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ScienceDirect
Biosensors and Bioelectronics 23 (2008) 1512–1518
BIOSENSORS & BIOELECTRONICS
www.elsevier.com/locate/bios

Ultra sensitive sensor with enhanced dynamic range for high speed detection of multi-color fluorescence radiation

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Received 29 October 2007; received in revised form 16 December 2007; accepted 3 January 2008
Available online 19 January 2008

Abstract

This paper describes design of the new ultra sensitive sensor system for fluorescence detection applications. System comprises two units: optical spectra separation unit and detection unit. Optical unit of the sensor performs spatial spectra separation of signal from the laser excited fluorescence, and resulting spectra is collected in the detection part of the system. Optical part is built using diffraction grating as spectra separation element. Detection part comprises 32-channel photomultiplier tube working in single photon counting mode with our 32-channel amplifier. Using single photon detection technique and specific signal processing algorithms for collected data, the proposed system allows to achieve unique combination of characteristics—high sensitivity, high detection speed and wide linearity dynamic range comparing to existing commercial instruments. DNA sequencing experiments with new sensor as detection device, and using two types of lasers (Ar-ion and Nd-YAG) were carried out, yielding sequencing traces which have quality factor of 20 for read lengths as long as 650 base pairs.

Keywords: Spectrometer; DNA sequencing; Fluorescence detection

1. Introduction

A number of extremely sensitive fluorescence detection techniques are available based on registering single photons (Papageorgas et al., 1999; Tiberg and Paulauskas, 1981; Evtyushenkov et al., 1982; Shelevoi, 1985) commonly referred to as the single photon detection (SPD) techniques. Because of their complexity and cost, in biomedical applications single photon detection techniques are mostly used for time resolved fluorescence spectroscopy or detection of single fluorescent molecules (Dovichi et al., 1984; Chen and Dovichi, 1996; Soper et al., 1992, 1993). A very important factor impeding the use of single photon detection is a relatively narrow linearity dynamic range of the available photon counting devices (typically order of 10^6 photocounts/s).

During last several years our research group has developed a number of single photon sensors with increased dynamic range and has demonstrated first DNA sequencing instruments possessing an ultra high sensitivity and a large detection dynamic range (Alaverdian et al., 2002; Gavrilov et al., 2003, 2006; Dhulla et al., 2005, 2007).

In this paper for the first time, we describe a novel single photon spectrometer, which is able to detect and identify compositions of multi-color fluorescent species with very high accuracy. The key component of the spectrometer—32-channel single photon detector developed by our group is a unique device with detection dynamic range of more than 20 bit and frame rate of 3300 frames/s. We shall also present a method which enables an accurate separation of fluorescent signals emitted by individual dyes. The dynamic range of the detector's channels reaches 10^7 photocounts/s and can be enhanced by a factor of 10 with the proposed signal processing algorithms.

2. Materials and methods

2.1. General description of the sensor

The sensor is developed to measure with single photon sensitivity radiation emitted by mixtures of minute amounts of

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doi:10.1016/j.bios.2008.01.015

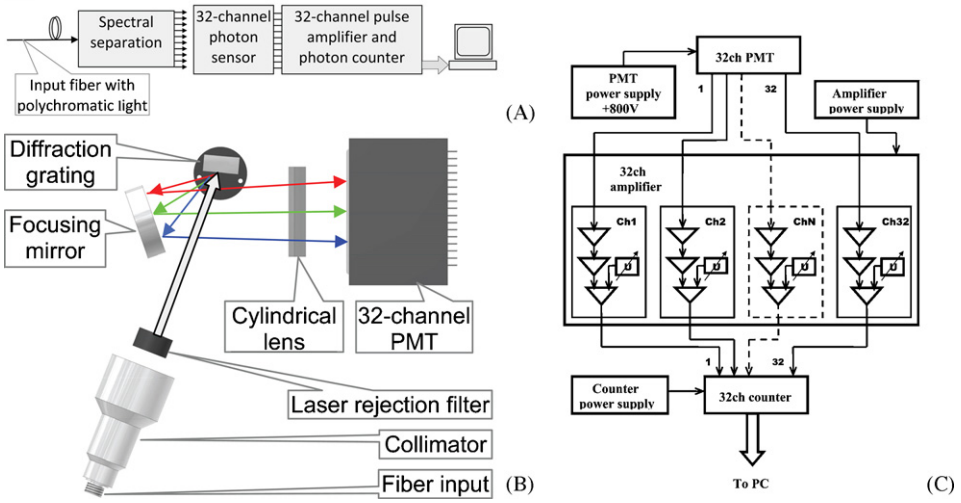

Fig. 1. Block diagrams of the sensor (A), spectral separation module (B) and 32-channel single photon detector (C).

multiple fluorescence dyes, and to determine very accurately a content of individual dyes in the dye mixture. Fig. 1A presents block diagrams of the sensor and its main modules. In the sensor, a polychromatic fluorescence collected by the input fiber passes through the spectral separation module (Fig. 1B) and the decomposed fluorescent signal illuminates photosensitive pixels of the 32-channel photosensor (32-channel PMT H7260-20, Hamamatsu, Japan). The received photons produce very short current pulses which undergo amplification and photon counting. The obtained photocount is transferred to computer for recording and data processing (see Section 2.3 for details).

2.2. Spectral separation module

This module performs separation and measurement of fluorescence in the range of wavelengths from 480 to 630 nm. The fluorescence comes to the spectrometer through the input fiber coupled to the collimator (F810SMA-543, Thorlabs Inc., NJ, USA) which produces a parallel polychromatic beam of ~10 mm diameter. The parallel beam passes through laser rejection filters and then undergoes separation on the diffraction grating (GR13-1850, Thorlabs Inc., NJ, USA) into constituent wavelength components. The separated monochromatic beams are focused onto channels of the 32-channel PMT (H7260-20, Hamamatsu, Japan) by the system comprising spherical mirror (CM254-075-G01, Thorlabs Inc., NJ, USA) and cylindrical lens (LJ1095L2, Thorlabs Inc., NJ, USA).

2.3. 32-Channel single photon detector and data format

Fluorescence detection in our 32-channel spectrometer is performed using 32-channel linear PMT array (H7260-20, Hamamatsu, Japan) in a single photon counting mode. Each channel of the PMT produces a stream of short (~1 ns) current pulses in response to an incident photon flux. The pulse amplitude ranges between 0.4 and 0.6 mA with corresponding peak voltage between 8 and 12 mV. In order to realize photon counting detection mode, we have developed a 32-channel pulse amplifier based on the surface mounted devices (SMD) technology and a 32-channel photon counter based on field programmed gate array (FPGA) technology. The amplifier comprises 32 identical pulse amplifying channels (35–40 dB gain, 1 GHz bandwidth) and 32 fast comparators having rise and a fall time of about 2 ns, which limits the minimum pulse width to approximately 4.5 ns. After amplification, a digitization (counting) of the amplified signal is performed by a home-designed 32-channel photon counter. The counting of the input photon pulses in each of 32 channels is performed by summation of pulses arriving to the channel input during the integration time intervals provided by a synchro-generator (minimum integration time is 0.3 ms, which corresponds to detection frame rate of ~3300 frames/s).

Data collected by the counter is transferred to a PC using standard IEEE 1284 Parallel Port Interface. The data is transferred in 105-byte frames using a binary format. Data frames consist of count values obtained for each of the 32 detection channels (3 bytes per channel). Each frame starts with a 6-byte header which includes the following fields: 1-byte counter type, 2-byte frame number and a 2-byte counting period length measured in milliseconds. The frame number contains the number of the current frame. The number is incremented by 1 for each following frame thus forming a rising sequence with overflow. The frame numbers serve as synchronization marks and are used by the data processing software to find data frames in the continuous data stream. Frame numbers are also used for verification of data integrity and for finding errors introduced by interference in the transmission line. A special software package performs the recording and the on-line visualization of the data transferred by the counter.

2.4. Channel cross-talk in multi-channel single photon detection system

First of all, we would like to note, that in the analyzed dyes mixtures proportion of different dye components may differs in orders of magnitude. Therefore, even a very small channel cross-talk in the sensor may cause an ambiguity in the