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Measuring fluorescence lifetime of a sample by fluorescent spectroscopy

Field of use Biotechnology, pharma, fluorescence detectors

Current state of technology Stage of Development: Available for demonstration

IPR status Patent(s) applied for but not yet granted

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Background

Fluorescence is the emission of light by certain substances, for example, fluorophores, such as dyes, or biomolecules, after they have been illuminated with light of specific excitation wavelengths. Measurements of the properties of fluorescent light emitted by various samples are used in a very wide range of applications. Just some examples are imaging of cell structures, tracking of antibodies and DNA sequencing in biology, detection of cancer cells in medicine and quality control in pharmaceutical production.

Description of the Invention

The fluorescence lifetime is most commonly determined with a timecorrelated single-photon counting (TCSPC), a method, where the time between the excitation pulse and the detection of individual fluorescence photons is measured. Intrinsic slowness of the TCSPC method and the induced photobleaching limit the applicability of measuring fluorescence lifetime in many potential fields, like in pharmacy, where high throughput of tested samples is required. Also, the TCSPC method requires complex and expensive instrumental setup.

A silicon photomultiplier, on the other hand, is a very fast photodetector, whose response to a single photon is faster than the fluorescence lifetime. Therefore, the shape of the electronic signal, i.e., the waveform, output by the silicon photomultiplier will follow the exponential decay of the fluorescence light resulting from a single pulse of excitation. If the resulting waveform is sampled with sufficient accuracy, the need for long accumulation of single-photon arrival times and also large excitation light intensities can thus be avoided. Excitation light with low intensities reduces the risk of photobleaching. Thus, the method allows to measure the fluorescence lifetime and, at the same time, avoids lengthy data acquisition and photobleaching of the sample.

Main Advantages

The main advantages of the method proposed over TCCSP:

- Cost-effective compared to common TCCSP technology
- Long accumulation of single-photon arrival times and also large excitation light intensities of TCCSP are improved
- Excitation light with low intensities reduces the risk of photobleaching