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# Characterization of biological materials by frequency-domain fluorescence lifetime measurements using ultraviolet light-emitting diodes

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**Abstract**

Recently developed deep-ultraviolet light-emitting diodes (UV LEDs) emit at wavelengths short enough to excitate fluorescence in most biological autofluorophores. We demonstrate the possibility of harmonical modulation of the output of group-III-nitride based UV LEDs ranging from 255 to 375 nm at frequencies up to 200 MHz. This enables the application of UV LEDs for frequency-domain fluorescence lifetime sensing with subnanosecond resolution. We report on measurements of fluorescence decay time in common biofluorophores (tyrosine, tryptophan, NADPH, NADH, collagen, DPA, elastin and riboflavin) using commercially available UV LEDs. We demonstrate the capacity of a multichannel LED-based frequency-resolved measurement technique to discriminate between *Bacillus globigii* and a variety of ambient interferants such as diesel fuel, paper, cotton, dust, etc.


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## 1. Introduction

Recent progress in ultraviolet (UV) optoelectronics [1] has resulted in the development of solid-state sources of light for fluorescence excitation in a variety of chemical and biological compounds. In particular, UV light-emitting diodes (LEDs) featuring AlGaN multiple-quantum-well active layer design have attained milliwatt cw output powers for wavelengths in the UVC region (250–280 nm) [2,3]. These wavelengths are short enough to excite fluorescence in proteins and are important for applications in medical and biological instruments. Due to their small dimensions and weight, low power consumption, stability and low cost, deep-UV LEDs are excellent candidates for many fluorescence sensor applications, such as point-of-care medical diagnostics or detection of hazardous biological compounds and agents.

Fluorescence measurement is a sensitive technique which allows for the detection of single molecules. Typically, biological recognition is based on the detection of natural autofluorophores such as nicotinamide adenine dinucleotide (NADH) and flavin enzyme cofactors, as well as antibodies and DNA sequences comprising aromatic amino acids (tyrosine, tryptophan and phenylalanine). When the intrinsic fluorescence of biomaterials is weak, various synthetic organic fluorophores can be used as probes and labels in biophysics and immunoassays [4,5]. Measurements of fluorescence intensity, decay time (lifetime), polarization and anisotropy, fluorescence resonance energy transfer and fluorescence quenching provide spectroscopic data allowing for the detection and recognition of various agents. Recently, deep-UV LEDs have become

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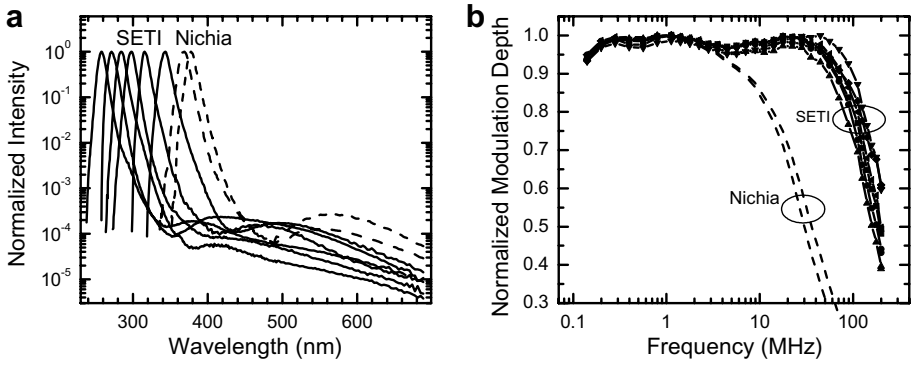


Fig. 1. (a) Electroluminescence spectra of SETI UV LEDs, with peak wavelengths at 258, 271, 282, 298, 315, and 343 nm (solid lines), and Nichia LEDs (368 nm and 378 nm, dashed lines). (b) Modulation characteristics of SETI LEDs with cut-off frequencies of 110–170 MHz (solid lines) and of Nichia LEDs with cut-off frequencies of about 20 MHz (dashed lines).

commercially available within a variety of emission wavelengths that can be tailored to the fluorescence excitation of almost all biological objects [1,6–11].

In the present work, we demonstrate the possibility of output modulation of the commercially available UV LEDs emitting in the range from 255 to 375 nm at frequencies up to 200 MHz. It has enabled us to perform frequency-domain fluorescence decay-time identification of typical biofluorophores: tyrosine, tryptophan, reduced nicotinamide adenine dinucleotide phosphate (NADPH), NADH, collagen, dipicolinic acid (DPA), elastin and riboflavin. Finally, we demonstrate the possibility of discriminating between *Bacillus globigii* spores (a simulant of *Bacillus anthracis*, a hazardous biological agent) against a variety of typical airborne interferants with a multichanel UV LED-based frequency-resolved measurement technique.

## 2. Experimental

Commercially available UV LEDs developed by Sensor Electronic Technology, Inc. (SETI, USA) and Nichia Corp. (Japan) were tested for their spectral purity of emission and high-frequency modulation. Spectral purity and modulation characteristics of excitation sources are of crucial importance for fluorescence-spectra and frequency-domain fluorescence lifetime measurements. For AlGaN-based LEDs emitting in the range of 255–340 nm and InGaN-based LEDs emitting in the range of 365–375 nm, high-contrast emission spectra were resolved using a calibrated low-stray-light double monochromator (JY HRD1) and recorded using a photomultiplier operating in the photon counting mode (Hamamatsu R1463P). For the frequency-response measurements, the LEDs were driven using a bias tee (Picosecond 5547). The bias current was 20 mA and radio-frequency modulation of +5 dbm was provided by a high-power signal generator (Aeroflex IFR 2023A). The optical signal was recorded with a subnanosecond-risetime photomultiplier (Hamamtsu H6780-01) and measured with a radio-frequency lock-in amplifier (Stanford Research Systems SR844).

Emission spectra within a dynamic range of over 5 orders of magnitude for the LEDs driven at 20 mA are shown in Fig. 1a. The spectra peak at 258, 271, 282, 298, 315, and 343 nm for the SETI LEDs and at 368 and 378 nm for the Nichia LEDs, respectively. The full width at half magnitude of the line is from 10 to 12 nm for the SETI LEDs and from 11 to 13 nm for the Nichia LEDs, respectively. The emission line is asymmetric and a long-wavelength wing ranging throughout the entire visible spectrum is observed. The intensity of the far long-wavelength background constitutes about a $10^{-4}$th of that at the peak.

Modulation characteristics of the SETI and Nichia LEDs in the 25 kHz–200 MHz frequency range are shown in Fig. 1b. The SETI LEDs exhibit pronounced modulated emission up to the frequency of 200 MHz. The cut-off frequencies are from 110 to 170 MHz for the SETI LEDs and about 20 MHz for the Nichia LEDs. The modulation ability of the SETI LEDs is sufficient to measure the phase shifts between excitation and fluorescence waveforms for extraction of the fluorescence decay time in the subnanosecond range relevant to biofluorophore identification.

We have chosen SETI UV LEDs emitting at the optimal wavelengths of 340 and 280 nm for further measurements of biofluorophore fluorescence spectra and lifetimes. The fluorescence spectra were recorded using the same experimental setup as that described for the spectral investigation of LEDs. In the frequency-domain fluorescence lifetime measurement setup, the modulated UV LED excitation was collimated, short-pass filtered, reflected by a dichroic mirror and focused on the sample. Fluorescence from the sample was collimated by the same fused silica lens, passed through the dichroic mirror, filtered through a low-pass filter and focused on the cathode of the photomultiplier. The fluorescence signal was detected with low pass filters (LPF) transmissive in the range from 320 to 380 nm and from 420 to 480 nm for the 280-nm and 340-nm excitation, respectively. The electrical signal was split into ac and dc components by the bias tee and fed into a radio-frequency lock-in amplifier that measured the fluorescence phase and modulation depth [12].