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UV LED arrays at 280 and 340 nm for spectroscopic biosensing

K. Davitt[1], **Y.-K. Song**[1], **W. Patterson**[1], **A. V. Nurmikko**[*, 1], **Z. Ren**[2], **Q. Sun**[2], and **J. Han**[2]

[1] Division of Engineering, Brown University, Providence RI 02912, USA
[2] Department of Electrical Engineering, Yale University, New Haven CT 06520, USA

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We demonstrate the application of linear arrays of ultraviolet light emitting diodes (UV LEDs) at the wavelengths of 280 nm and 340 nm to the acquisition of fluorescence spectra from single particles in real-time. A compact optical system and custom electronics are employed to produce a compact, standalone biosensor. The detection sensitivity of the UV LED system is established using the biofluorophores tryptophan and NADH, and the capability of the sensor to sort particles based on weak spectra is demonstrated using micron-sized polystyrene latex (PSL) spheres.

1 Introduction

Improvements in III-nitride material growth and resulting device performance has yielded state-of-the-art UV LEDs that have sufficient output powers so as to be useful in fluorescence-based bio-sensors. Much interest has recently been invested in systems using UV LEDs to excite native fluorescence from bacterial spores in air [1], however, fluorescence sensors encompass both systems examining native biofluorophores and those using artificially labelled molecules to enhance discrimination between particles. Alternatively, these sensors can be subdivided by the detection method or by the method of sample delivery – static or in flow, in aerosol form, on a solid substrate or in a fluidic environment. Semiconductor light emitters offer several advantages over the solid-state lasers that have traditionally been used in these types of sensors, most notably in size, wavelength tunability and cost. Here we propose a sensor employing UV LEDs and demonstrate its operation by acquiring fluorescence spectra from single airborne particles, however, we propose that this system concept is flexible so as to be portable to other sample environments.

2 UV LED array design and performance

The reduced total output power and spatial incoherence of UV LEDs with respect to solid-state lasers represents a challenge for sensor design. On the other hand, LEDs offer the advantage that the illumination pattern can be easily tailored to accommodate the task at hand. We employ a linear array of individually addressable LEDs whose elements are fired in rapid sequence so as to essentially continuously illuminate a particle as it flies by the array. This scheme has several advantages over the use of a single element device, notably an increase in the total energy delivered to the particle, achieved both by extend-

[*] Corresponding author: e-mail: Arto_Nurmikko@brown.edu, Phone: +1 401 863 2869, Fax: +1 401 863 9120

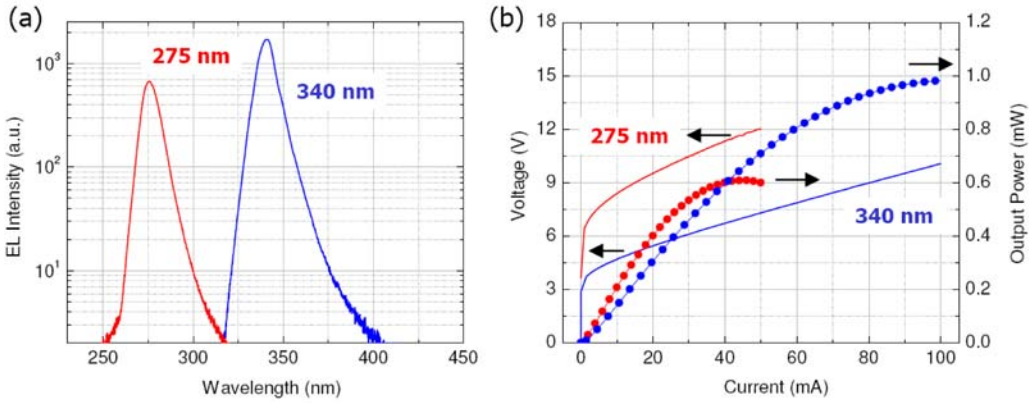

Fig. 1 (online colour at: www.pss-a.com) Characterization of UV LED array performance; (a) electroluminescence and (b) dc light–current–voltage for a single LED element as measured directly off the die.

ing the excitation time as well as enabling the single UV LED elements to be driven in a short pulse, high current injection regime.

The 275 nm (340 nm) LEDs are MOCVD grown ternary AlGaN (quaternary AlInGaN) MQW heterostructures grown on sapphire substrates. Details of the epitaxial layer design have been published elsewhere [2, 3], as has the description of the array geometry, packaging and microelectronic fabrication technique [4]. Briefly, the array consists of 32 individual UV LEDs, each with an optical aperture 200 μm wide and 50 μm high, and sharing a common n-electrode. Figure 1(a) shows the log scale electroluminescence spectrum of the devices used to acquire the results presented in Section 4. Figure 1(b) contains typical dc light–current–voltage traces from single LED elements before flip-chipping, where the power is measured with a UV-enhanced silicon photodiode placed directly on the sapphire backside of the LED. In the demonstration, each element of the 275 nm (340 nm) array is pulsed for approximately 70 usec under 20 mA (40 mA) injection.

A recent investigation into bulk aluminium nitride (AlN) as an alternative substrate for growth of high aluminum-content devices has yielded sub-300 nm LEDs with output powers approximately 4 times that of similar devices on sapphire and do not exhibit a thermal rollover up to current densities of 2 kA/cm^2 [5]. In addition to an increased output power, these devices have clean electroluminescence spectra, which relaxes the requirements on the excitation filter used in fluorescence experiments. For these reasons, UV LEDs grown on bulk AlN represent a promising improvement for fluorescence biosensors, but are not yet employed in this demonstration due to the limited size of uniform AlN substrates.

3 Optical and electrical system design

Figure 2 illustrates the optical apparatus into which the UV LED array is inserted. The array is focussed to the particle trajectory by 1:1 imaging optics, and the resulting fluorescence is collected at a right angle to the illumination using a compact spectrometer composed of a single lens, a scatter-blocking filter, a UV transmission grating and a 32-anode photomultiplier (PMT). Scatter at the LED wavelength is collected simultaneously by a simple PMT and filter. In order to synchronize the sequential LED flashing with the arrival of a particle, a red laser diode is focussed to the particle jetstream immediately prior to the LED image and scatter at this wavelength is detected by a trigger PMT.

Located downstream from the fluorescence optics is a miniature particle deflector. When energized by a current pulse, the solenoid-based deflector generates a burst of nitrogen gas which removes a particle from the jetstream and deposits it on the vertical portion of the glass substrate illustrated in Fig. 2(a). A set of custom electronics has been developed to drive the LED array, collect the numerous optical input signals and to analyze the acquired single-particle spectra in real-time so as to trigger the deflector as the

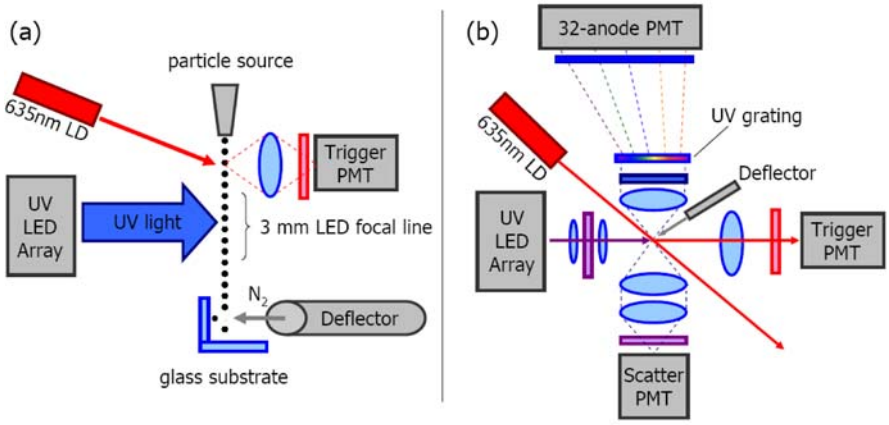

Fig. 2 (online colour at: www.pss-a.com) (a) Side and (b) top view schematics of the compact optical system for single-particle fluorescence sensing using UV LEDs.

offending particle flies by. The core of the electronics is a commercial product (Vtech Engineering Corp.), which has been modified by several additional electronics boards for this specific task [6]. The total optical system occupies a footprint of 25 cm × 35 cm; a size that is commensurate with the compact nature of the LED source. We note that although this demonstration uses aerosolized particles, the optical and electrical systems are designed to accommodate microfluidic environments in general.

4 System demonstration

Biological materials contain key constituent molecules such as the amino acids tryptophan and tyrosine, and reduced nicotinamide adenine dinucleotide (NADH), which exhibit characteristic fluorescence spectra that enable discrimination between particles of biological and non-biological origin, and perhaps between species of the former. These molecules have significant absorption only below 300 nm, with the exception of NADH which has a secondary maximum centred on 340 nm, hence UV LEDs at 280 nm and 340 nm are desired for native bio-fluorescence sensors.

Figure 3(a) shows fluorescence spectra acquired from 70 µm tryptophan-doped water droplets illuminated by a 275 nm LED array. The illustrated spectra are averaged over 100 nominally identical droplets,

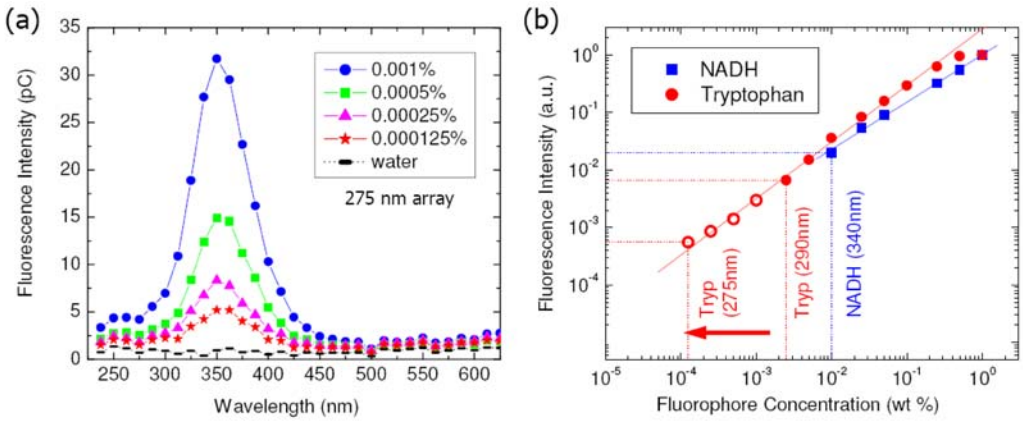

Fig. 3 (online colour at: www.pss-a.com) System detection sensitivity; (a) average fluorescence spectra from single droplets of different tryptophan concentrations (by wt%), and (b) detection limit of tryptophan/NADH under illumination from different LED wavelengths.

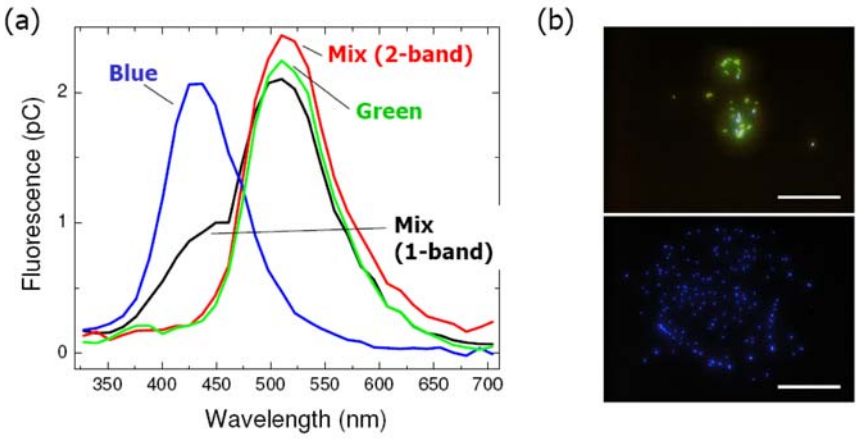

Fig. 4 (online colour at: www.pss-a.com) Demonstration of particle separation capability; (a) average flagged fluorescence spectra, and (b) epifluorescence images of deflected (green) and undeflected (blue) particles. Scale bar corresponds to 100 μm.

where each single spectrum was acquired within 2 ms, corresponding to the fly-by time of the particle past the array. In order to characterize the detection limit, Fig. 3(b) shows the integrated fluorescence signal from the system employing a 340 nm LED array to detect NADH and employing a sub-300 nm LED to detect tryptophan. The open circles show the improvement in detection limit compared to our previously published report [6]. Computing the detection limit in terms of number of molecules, the system is shown to be able to detect approximately 3×10^8 tryptophan or 10^{10} NADH molecules. Based on the dry weight and tryptophan concentration of a typical *bacillus globigii* spore [7], this is commensurate to between 1 and 5 individual spores.

In order to demonstrate the sensor's capability to spectrally identifying and sort single particles from a background of non-identical fluorescing particles, water droplets were doped with a low concentration mixture of blue (1 μm diameter) and green (2 μm) fluorescing PSL spheres. PSL, as opposed to mixtures of tryptophan and NADH, are useful characterization tools as they are present in discrete quantities and can be counted to verify results. For the data shown in Fig. 4, a 340 nm LED array was operated at a modest injection current of 20 mA and the electronics were programmed with an algorithm to isolate either blue or green beads using a simple threshold level of 1 pC and two wavelength bands, one centred at 525 nm and the other at 435 nm. Results of the two-band algorithm were used to trigger the aerodynamic deflector and Fig. 4(b) is an epifluorescence image of the separated particles. Counting deflected particles, yields nominally 180 green and 8 mistakenly removed blue ones from a set of 5000 droplets.

It has been shown that a compact optical system employing state-of-the-art UV LED arrays at 275 nm and 340 nm is able to detect and physically sort particles based on weak fluorescence spectra acquired in real-time. The detection sensitivity of the system is promising for the detection of a small quantity of biological material, on the order of a few individual bacterial spores, from native fluorescence alone. The system has been demonstrated using airborne micro-particles, and work is ongoing to apply a similar emitter array and spectroscopic detection scheme to a microfluidic environment, with the ultimate goal of producing a chipsize UV LED-based biosensor.

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References

[1] J. Carrano et al., Unattended Ground Sensor Technologies and Applications IV, Proc. SPIE **4743**, 233 (2002).
[2] W. Sun et al., Jpn. J. Appl. Phys. **43**(11A), L1419 (2004).

[3] S. R. Jeon et al., Jpn. J. Appl. Phys. **43**(11A), L1409 (2004).
[4] K. Davitt et al., Opt. Express **13**(23), 9548 (2005).
[5] Z. Ren et al., phys. stat. sol. (c) **4**(7), 2478 (2007), this conference.
[6] K. Davitt et al., Aerosol Sci. Technol. **40**, 1047 (2006).
[7] W. G. Murrell, in: The Bacterial Spore, edited by G. W. Gould and A. Hurst (Academic Press, London, 1969).

