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High-power blue/UV light-emitting diodes as excitation sources for sensitive detection

With advances in III-V nitride manufacturing processes, high-power light-emitting diode (LED) chips in the blue and UV wavelengths are now commercially available at reasonable cost and can be used as excitation sources in optical sensing. We describe the use of these high-power blue and UV LEDs for sensitive fluorescence detection, including chip-based flow cytometry, capillary electrophoresis (CE), and single-molecule imaging. By using a blue LED with a focusable power of ~ 40 mW as the excitation source for fluorescent beads, we demonstrate a simple chip-based bead sorter capable of enriching the concentration of green fluorescent beads from 63% to 95%. In CE experiments, we show that a mixture of analyte solution containing 30 nM 6-carboxyrhodamine 6G and 10 nM fluorescein can be separated and detected with excellent signal-to-noise ratio (~ 17 for 10 nM fluorescein) using the collimated emission from a blue LED; the estimated mass detection limit was ~ 200 zmol for fluorescein. We also demonstrated ultrasensitive fluorescence imaging of single rhodamine 123 molecules and individual λ -DNA molecules. At a small fraction of the cost of an Ar⁺ laser, high-power blue and UV LEDs are effective alternatives for lasers and arc lamps in fluorescence applications that demand portability, low cost, and convenience.

Keywords: Capillary electrophoresis / Fluorescence / Lab-on-a-chip / Light-emitting diode / Miniaturization / Single-molecule imaging
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1 Introduction

Fluorescence detection has evolved into the method of choice for many applications in biotechnology, owing to its inherent sensitivity, noninvasiveness, ease of implementation, and the commercial availability of many well-developed fluorescent dyes and labeling schemes for biological studies. Some examples of the widespread usage of fluorescence detection include capillary electrophoresis (CE) [1–5], enzyme assays [6–10], DNA sequencing and fragment sizing [11], antibody detection and screening [4], bead-based immunoassays [12, 13], cell sorting [14–16], enzyme-linked immunosorbent assay (ELISA) [17], cellular microscopy and imaging [18, 19], and single-molecule detection [20–27]. In most fluorescence applications, either a laser or a high-powered white-light source equipped with appropriate filters is used for exciting fluorescence. Although these standard excitation sources deliver sufficient power and suitable optical characteristics for fluorescence detection, they are often expensive and bulky, especially in the blue wavelength region (~ 450 – 500 nm), in which an Ar⁺ laser

or an arc lamp source (mercury or deuterium typically) is often required. GaN-based blue laser diodes are commercially available, but the price of high-power units can be even higher than that of Ar⁺ lasers of comparable power. Excitation in this wavelength range is particularly versatile because of the commercial availability of a wide range of suitable dyes, including fluorescein-based dyes [18] and green-fluorescent proteins (GFPs) [28]. In contrast, the available dyes in the red region are fairly limited and more expensive, although economical and compact laser sources in this region of wavelength (e.g., red diodes and HeNe lasers) are readily available.

Since the report of the first GaN p-n junction blue light-emitting diode (LED) in 1989 [29], an intense amount of research effort worldwide has been devoted toward developing robust manufacturing processes to commercialize LEDs in the blue and UV range. After substantial effort in overcoming growth issues, such as film-substrate lattice mismatching, thin-film purity, and control of crystallinity, the first InGaN/AlGaIn LEDs became commercially available in 1994 from Nichia Chemical Industries [30–32]. The first blue LED had an output power of 2.5 mW (at 40 mA input current), which was hailed as the highest at the time [31]. Now, in the second decade of commercialization, it is possible to purchase individual resin-encapsulated blue LEDs from retail stores inexpensively ($\sim \$5$ US). Although these LEDs are bright enough for illuminating keyholes in the dark, they are by no means representative of the state-

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Abbreviations: PDMS, polydimethylsiloxane; PMT, photomultiplier tube

of-the-art in high-power LED development, which has been driven in part by applications that require intense illumination, such as outdoor traffic lights [30] and displays.

Here, we report our experience in sensitive fluorescence detection using the latest high-power LEDs in development by Cree Research (Durham, NC, USA). These LEDs can be purchased as diced chips (XBRIGHT™ Power Chip; C470-XB-900 for blue, C405-XB-900 for UV). Unlike the LEDs sold in electronic stores, whose radiant flux seldom exceeds 1 mW, Cree's XBRIGHT™ Power Chip's radiant flux (at $I_{\text{forward}} \sim 350$ mA) is nominally 150 mW for blue LEDs, and 250 mW for UV LEDs. Unlike previous manufacturing efforts, Cree employs SiC rather than sapphire as the substrate material, resulting in lower defect density [33]. The principal difficulty in using these LED chips is to ensure adequate heat removal in the design of the packaging. Traditional resin-dip method of packaging does not work with these chips since resins cannot dissipate heat rapidly enough. Standard clear silicone encapsulant used to protect the chip and the contact wires also turns brown within 24 h of use due to intense UV emission and high operating temperature. To address this issue we have designed and constructed our own packaging to ensure adequate heat dissipation so the LED can be operated at high power.

Unlike laser emission, LED emission is spatially incoherent and nonmonochromatic. To achieve proper focusing and the desired spectral output, considerable power loss should be expected in using LEDs as the excitation source. Prior to the availability of high-power LEDs, such power loss would have significantly reduced the detection sensitivity, although that has not thwarted recent attempts of using blue or UV LEDs as an inexpensive excitation source, which included efforts in both chip-based microfluidics platform as well as non-chip-based systems [34–44]. In exploring the applicability of high-power LEDs for sensitive fluorescence detection, we examined several systems, including chip-based flow cytometry, CE, and single-molecule imaging.

2 Materials and methods

2.1 Characterization of LEDs

The LED chips were custom die-bonded and wire-bonded to a TO-5 header (Sinclair Manufacturing Company, Chartley, MA, USA) to meet heat dissipation requirement. A glass collimation lens cap (Schott North America Manufacturing, San Clemente, CA, USA) was placed atop the header, with the lens located at ~ 6 mm above the LED chip. Additional heat sinks were installed to enhance heat transfer. Custom-built regulated DC power supplies were constructed with parts available from commercial electronics component catalogs (e.g., Digikey or Newark Electro-

tics) to ensure adequate current supply. A spectrophotometer (Ocean Optics USB2000, Dunedin, FL, USA) was used to measure the LED emission spectra. The emission peaks were calibrated to the emission maximum of a 100 μM fluorescein in 10 mM sodium borate: in basic solution, it is known that only the dianion form of fluorescein exists [45, 46], which has emission maximum at

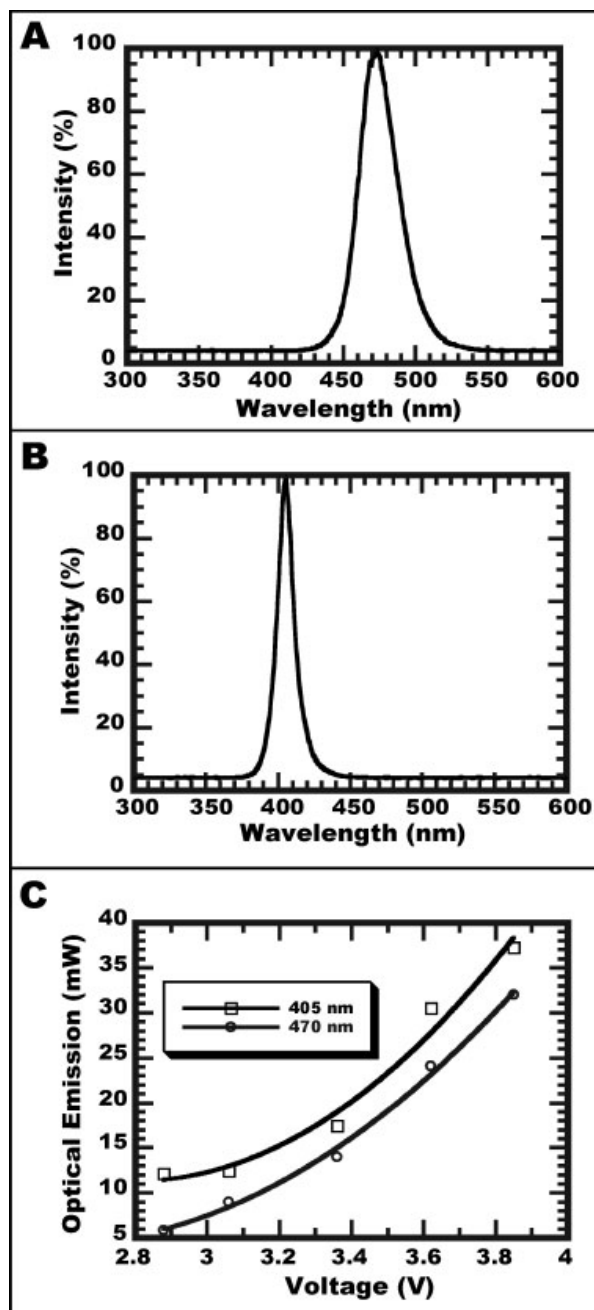


Figure 1. (A) Emission spectrum of a high-power blue LED. (B) Emission spectrum of a high-power UV LED. (C) Optical power output of LEDs as a function of operating voltage.

520 nm [35]. The optical power of LED emission was characterized with a Nova optical power meter (OPHIR Optonics, Wilmington, MA, USA) by placing the meter as close to the collimation lens as physically possible. The nominal background fluctuation of the meter was 0.1 mW. Figure 1 summarizes the typical LED performance characteristics using the spectrophotometer and the power meter.

2.2 Chip-based flow cytometry

A flow cytometer chip was constructed out of polydimethylsiloxane (PDMS) using rapid-prototyping procedures as described in [47]. The circular inset of Fig. 2A shows the arrangement of the microchannel, which was in the form of a T-junction. The widest section of the channel in each branch (near the reservoirs) was 150 μm in width, tapered down to 15 μm over a transition length of 100 μm , and then remained at 15 μm wide for 1000 μm in length prior to the T-intersection. The height of the channels was 15 μm throughout the chip. Prior to use, the PDMS channel was oxidized in oxygen plasma at high-power setting (29.6 W applied to the RF coil at a nominal O_2 pressure of 200 mtorr) for 2 min using a Harrick PDC-001 RF Plasma Cleaner (Harrick Scientific, Ossining, NY, USA). This oxidized piece was sealed to a 2" $\times$ 3" glass microscope slide, which was precoated with a thick layer of PDMS and was oxidized along with the PDMS channel. The sealed channel was then immediately filled with 10 mM sodium borate buffer solution. Two types of fluorescent beads were used in bead-sorting experiments: G0500 green fluorescent spheres (nominal diameter 5 μm ; $\lambda_{\text{ex}} \sim 468$ nm, $\lambda_{\text{em}} \sim 508$ nm) and 36-2 Red fluorescent beads (nominal diameter 7 μm ; $\lambda_{\text{ex}} \sim 542$ nm, $\lambda_{\text{em}} \sim 612$ nm). Both were purchased from Duke Scientific (Palo Alto, CA, USA). The beads were diluted in 10 mM borate solution and mixed at a ratio of 5:3 green to red beads ($\sim 7 \times 10^7$ green beads/mL; 4×10^7 red beads/mL).

Figure 2A shows the arrangement of the optics we used in chip-based bead-sorting: A PDMS bead sorter was placed on a microscope stage, illuminated with a focused light spot by using combinations of plano-convex and plano-concave lenses and a dichroic. An extralong working distance 20 $\times$ objective was used for the final focusing of the light from the LED. The fluorescence emission was detected using a Hamamatsu R928 photomultiplier tube (PMT), whose output was measured with a Keithley 6485 picoammeter. Three negative-polarity high-voltage power supply modules were used to control the direction of electroosmotic flow (EOF). A computer program was written in LabView environment to control the power

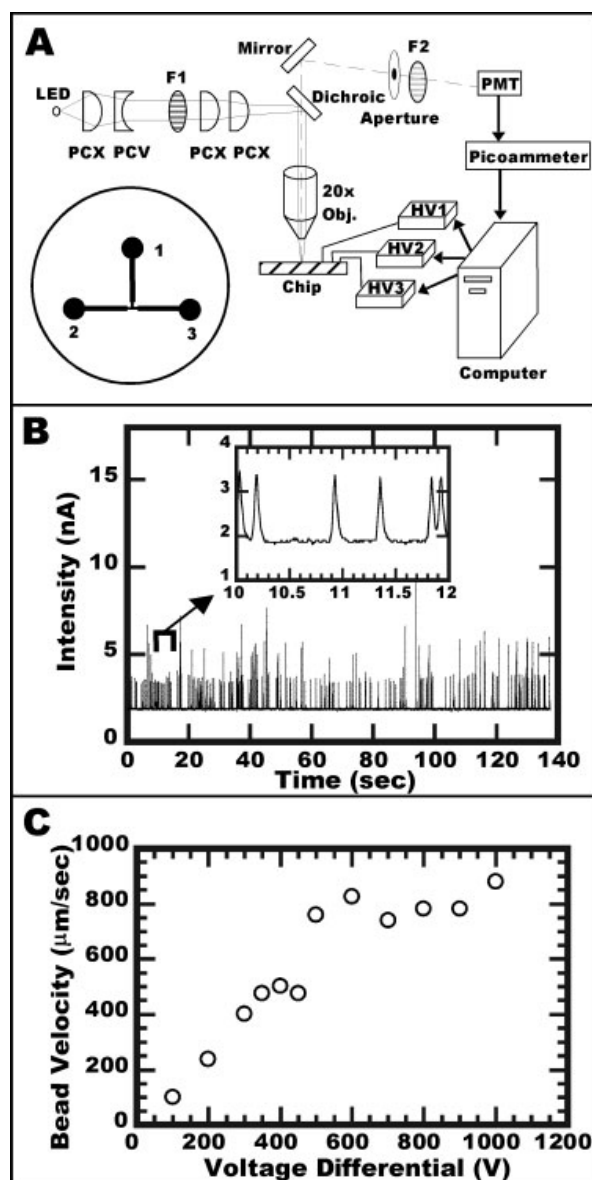


Figure 2. (A) Experimental setup for chip-based flow cytometry. Circular inset: schematic of the microchannels used for bead sorting (not drawn to scale). PCX, plano-convex; PCV, planoconcave; PMT, photomultiplier tube; F1, excitation filter (Omega 470DF35); F2, emission filter (Chroma HQ515/30m). (B) PMT trace of green fluorescent beads in 10 mM sodium borate buffer, driven by -800 V differential (Reservoir 1 = 0 V; Reservoir 2, 3 = -800 V). Inset shows the discrete peaks detected. (C) Velocity of beads in T-junction as a function of driving voltage.

supply outputs, and hence the bead trajectory, based on the detected fluorescence signal. To sort different color beads, a "threshold" algorithm similar to the one used by Fu *et al.* [15, 16] was implemented to discriminate be-

tween rhodamine beads, whose fluorescent emission was nearly completely blocked by the emission filter, from fluorescein beads, by comparing the voltage trace from the PMT to a threshold voltage. The program by default directed the flow into Reservoir 3 (waste) with the following voltages: $V_1 = 0$ V, $V_2 = -320$ V, $V_3 = -600$ V. If the PMT signal reached certain threshold voltage (e.g., when a fluorescein bead was detected), the program directed the flow into Reservoir 2 (product) with the following voltages: $V_1 = 0$ V, $V_2 = -600$ V, $V_3 = -320$ V. The travel time of the bead and the timing of the voltage in each branch of the T-channel required careful optimization because sorted beads that had not yet reached the intended reservoirs were susceptible to flow reversal and might be directed into the wrong reservoir. In addition, at high sorting velocities, bead clogging at constrictive points can be a serious problem. To address this clogging problem, the flow was reversed every 60 s (directing back to Reservoir 1) for an 8-s duration to dislodge the beads in the constriction ($V_1 = -1800$ V, $V_2 = -900$ V, $V_3 = 0$ V), after which the sorting was resumed.

2.3 Capillary electrophoresis

A custom-built CE setup was constructed as depicted in Fig. 3A. The optical setup consisted of a high-power blue LED as the fluorescence excitation source, a stationary-mounted microscope objective, filters and lens for collimation and wavelength selection, and a Hamamatsu R928 PMT for fluorescence detection. A 30 kV high-voltage power supply (Acopian Technology, Easton, PA, USA) was used to apply voltage across the capillary. A fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) with 50 μ m inner diameter (ID) was mounted onto an x-y translation stage to allow adjustment of the position of the capillary relative to the focal point of light from the LED. A 50 mM sodium borate solution, prepared with Milli-Q water and filtered through a 0.2 μ m particulate filter, was used as the buffer throughout the experiment. Prior to introduction of new analyte, 1 M NaOH was injected hydrodynamically into the capillary to condition the inner surface, followed by repeated flushing with the buffer solution. The analyte consisted of a mixture of 6-carboxyrhodamine 6G (C-2213; $\lambda_{ab} \sim 520$ nm; $\lambda_{em} \sim 546$ nm) and fluorescein standard (F-1300, $\lambda_{ab} \sim 490$ nm; $\lambda_{em} \sim 514$ nm) in a borate buffer at a 3:1 ratio in molar concentration. Both dyes were from Molecular Probes (Eugene, OR, USA). The plug of analyte was injected by dipping the end of the capillary in the analyte mixture for ~ 30 s at 1 cm above the waste reservoir and then the capillary was returned to the buffer reservoir.

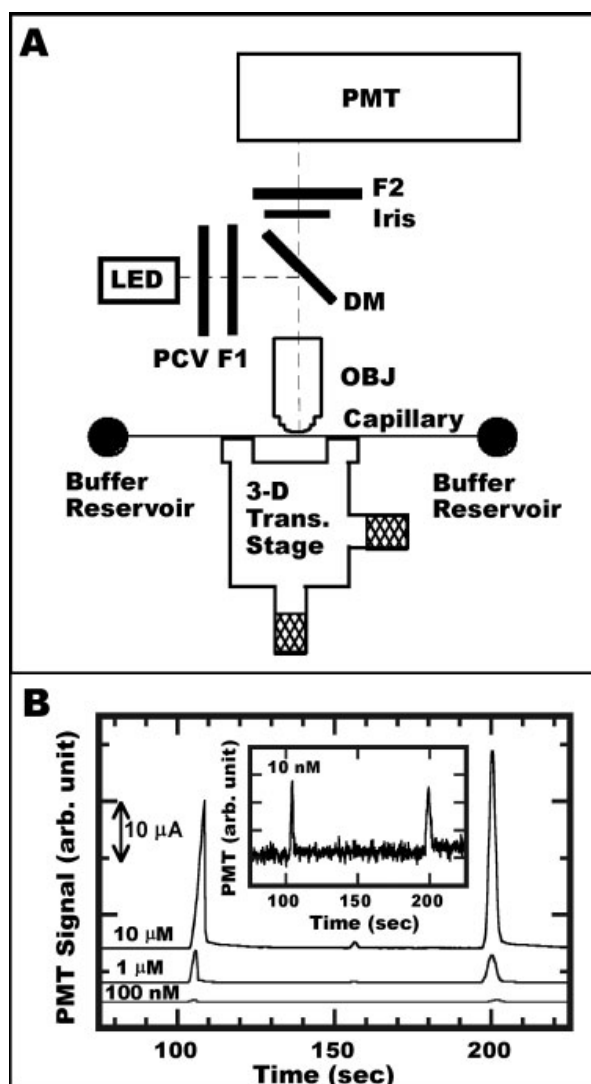


Figure 3. (A) Optical arrangement for CE experiment. PCV, planoconvex lens; F1, excitation filter (Omega XF1073); DM, dichroic mirror (Omega XF2010); F2, emission filter (Omega XF3086); OBJ, objective. (B) Electropherograms from separation of 6-carboxyrhodamine 6G (108 s) and fluorescein (200 s) at various dilutions (concentration of fluorescein at 10 μ M, 1 μ M, 100 nM, and 10 nM (inset)) in 50 mM sodium borate buffer at a field strength of 300 V/cm. A fused-silica capillary (50 μ m ID, 27 cm in length) was used for separation. Inset: electropherogram from a mixture of 30 nM carboxyrhodamine: 10 nM fluorescein.

2.4 Single-molecule imaging

The optical arrangement was similar to that used in our previous publications on the optical trapping and imaging of individual λ -DNA molecules [27, 48]. Instead of using an Ar⁺ laser as the excitation source, however, a high-

power blue LED was used. We used additional filters and collimation lenses to compensate for the nonmonochromatic and incoherent nature of the emission from LEDs. As shown in Fig. 4A, epifluorescence detection of single molecules was performed on an inverted microscope (Nikon TE2000U, Melville, NY, USA) equipped with a $100\times$ 1.45 NA Nikon objective and sensitive CCD cameras (Andor Technologies Ixon 87 (South Windsor, CT, USA) and Photometrics Cascade 650 (Roper Scientific, Tucson, AZ, USA)). Imaging of single rhodamine 123 molecules was achieved by summing ten 1.5 s exposures (binning 2×2) to produce a 14-bit digitized image (Fig. 4B). ImageJ software (NIST) was used to enhance the image quality and to produce the surface plot in Fig. 4C. To calculate the signal-to-noise ratio (S/N), the maximum intensities of ten molecules were averaged and then divided by the standard deviation of the background noise, which was calculated from a 100-pixel linescan through a portion of the image where no molecules were detected. The sample was prepared by placing 1 drop (20 μ L) of a 30 pM solution of rhodamine 123 (Molecular Probes R-302) in Milli-Q water onto a glass coverslip. To reduce surface contamination, the coverslip was boiled in a solution of 3:2:1 $\text{H}_2\text{O}:\text{NH}_4\text{OH}:\text{H}_2\text{O}_2$ for >1 h, rinsed in Milli-Q water, then dried under a stream of N_2 immediately before use. To ensure that the observed fluorescence signals were from single molecules, an Ar^+ laser at 488 nm (Coherent, Santa Clara, CA, USA) was used to provide ample illumination that allowed visualization of the molecules at 100 ms resolution. At this time resolution, blinking of the fluorescent spots could be observed, which indicated that the images were of single molecules (data not shown).

3 Results and discussion

3.1 Characterization of LEDs

Figure 1A shows the emission spectrum of a blue LED, which has a maximum at 475 nm with a full-width at half-maximum (FWHM) of ~ 34 nm. There is slight tailing past 500 nm, accounting for approximately 7% of the total spectral emission, which must be filtered out to avoid overlapping with the fluorescence emission from fluorescein. Figure 1B shows the emission spectrum of a UV LED, which centers at 405 nm, and has a narrower FWHM than the blue LED (~ 16 nm). Figure 1C plots the optical power output as a function of applied voltage, which shows a typical operating output of ~ 40 mW. Although this optical power is comparable to the output of an air-cooled Ar^+ laser, one must keep in mind that LED emission is nonmonochromatic and spatially incoherent. Consequently, spectral filtering and inefficiency in collimation can result in substantial reduction in the power that is delivered to the sample. Our measurement is substantially lower than Cree's nominal radiant flux rating (150 mW for blue LED) because the measurement was not taken with a spectroradiometer, which would have been able to measure the output over the entire 2π steradian. Instead our measurement was taken with a collimation lens at about 6 mm above the chip, which prevented photon collection in certain steradian, omitting in particular the emission from near the edge of the chips. Therefore, our measurement represents the amount of power from the LED that can be realistically collected, collimated, and focused.

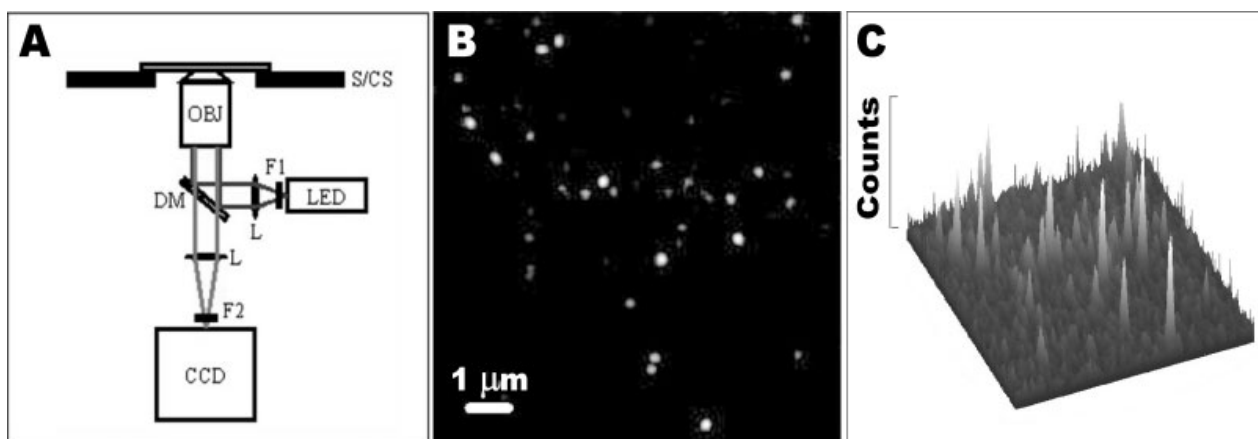


Figure 4. (A) Optical configuration for imaging single molecules with the blue LED. S/CS, stage/coverslip; OBJ, objective; DM, dichroic mirror (Chroma 488/1064pc); L, lens; F1, excitation filter (Omega XF1073); F2, emission filter (Chroma HQ550/100). (B) Image of single rhodamine 123 molecules on the surface of a glass coverslip. (C) Three-dimensional intensity plot of the single dye molecules imaged in (B).

It is also important to note that the temperature of the chip can affect the radiant flux as well as the emission profile: At higher temperatures the amount of light emitted by LEDs decreases because nonradiative recombination processes (e.g., contribution from carrier leakage assisted by structural defects [49]) can be significant; concurrently the emission spectrum can also shift slightly towards longer wavelengths. We have not systematically optimized our design for heat dissipation to reduce the temperature of the junction, and it is possible that with the use of larger heat sinks and with additional thermoelectric cooling the emission profile can be narrowed and even more radiant flux can be generated.

3.2 Chip-based flow cytometry

Conventional flow cytometers have been used for cell sorting in a broad range of biological research and clinical applications [50]. For example, flow cytometers are now increasingly used in applications, such as the determination of viral load [51], the diagnosis and monitoring of autoimmune disorders [52] and carcinoma [53], and the screening of cells for stem-cell transplantation [54, 55]. Recently, microfabricated flow cytometers have been demonstrated to potentially become a high-sensitivity, disposable, and low-cost alternative to conventional flow cytometers, capable of rapidly sorting and recovering live fluorescent *Escherichia coli* cells [14–16] as well as fluorescent beads of different emission wavelengths [16]. With increasing interests in attaching μm -sized beads with antibodies for protein screening and biochemical assays, the ability to rapidly distinguish and sort fluorescent beads is expected to play a key role in disease diagnostic and monitoring. Here, we demonstrate the suitability of high-power LEDs for such applications.

Figure 2B is a PMT trace showing the detection of individual green fluorescent beads as they travel through the imaging spot. Because of the excellent fluorescence uniformity of the beads, the PMT intensity from individual moving beads was highly reproducible, as can be seen in the inset of Fig. 2B. By calibrating the peak width against the image spot size ($\sim 113\ \mu\text{m}$ in diameter), we estimated the bead velocity as a function of applied voltage (Fig. 2C). As expected, the velocity of the bead increased with the applied voltage. This increase is nonlinear because of the complex interplay between EOF, electrophoresis, and hydrodynamic transport of the beads.

To verify the performance of bead-sorting, five consecutive runs were analyzed. On average, the concentration of fluorescein beads was enriched from 63% in Reservoir 1 (source) to 95% in Reservoir 2 (product). Because of the large detection area, multiple beads may be

detected simultaneously that results in intensity summation in discrete levels. This occurrence poses a potential error in sorting, because if red beads were in close vicinity to a green bead they would both be directed to the product reservoir. In that case, the particular threshold algorithm we used was blind to the red beads. The achieved sorting accuracy can benefit from a more sophisticated discrimination algorithm, such as incorporating slope detection to assist in identifying finer intensity features.

3.3 Capillary electrophoresis

Capable of resolving components of complex mixtures of analytes with high resolution, CE is an extremely sensitive analytical technique. As commercial manufacturers of CE instrumentation continue to improve on automation and reproducibility, CE has now gained wide acceptance in clinical chemistry [56, 57] and related areas such as toxicology, forensic science, and pharmacology [58]. In CE, the most sensitive detection technique is laser-induced fluorescence (LIF): with appropriate fluorophores, LIF is capable of detecting the presence of a single molecule, and frequently detection limits in the attomole range are quoted for commercial CE setups. However, the excitation lasers used in LIF are sometimes the single most expensive and bulky component in commercial instrumentation. In this context, high-power blue/UV LEDs may be excellent alternatives to traditional laser sources.

Although the spatial coherence characteristic of lasers permits tight focusing ($<1\ \mu\text{m}$) that is important for certain applications, most fluorescence detection performed in microfluidics and separations does not require such a tight focal spot. Because the dimension of the channels in these applications is typically tens of micrometers in width, most analytes would not even transit such a small focal volume. For such applications, the spatially incoherent emission from LEDs does not represent a significant drawback. With optimized collimation, one can expect $\sim 500\ \text{W}/\text{cm}^2$ illumination (with a typical spot diameter of $\sim 100\ \mu\text{m}$), which is below the threshold for optical saturation and photobleaching. Figure 3B shows the electropherogram from separating carboxyrhodamine (at $\sim 108\ \text{s}$) and fluorescein (at $\sim 200\ \text{s}$) using CE. To evaluate the detection sensitivity based on LED excitation, Fig. 3B shows four sets of data that correspond to different levels of dilution. The top trace (highest intensity) corresponds to the separation of the stock mixture, which consisted of $3 \times 10^{-5}\ \text{M}$ carboxyrhodamine: $1 \times 10^{-5}\ \text{M}$ fluorescein; middle trace corresponds to a 10-fold dilution ($1\ \mu\text{M}$ fluorescein) of the stock, and the lowest intensity trace corresponds to a 100-fold dilution ($100\ \text{nM}$ fluorescein). The S/N ratio was ~ 17 for fluorescein at 1000-fold dilution ($10\ \text{nM}$ fluorescein, see

inset), which was more than sufficient for unambiguous peak assignment. Because 10 nM refers to the concentration of the fluorescein prior to injection into the capillary, the actual concentration after separation at the detection spot is more dilute. Based on the original concentration and an estimated plug volume of 0.1 nL (plug length calibrated with PMT signal and measured EOF velocity), the mass detected in this case corresponded to ~ 1 amol, which places the detection limit ($S/N = 3$) at ~ 200 zmol of fluorescein. This detection limit may be further improved with more sophisticated and optimized optical arrangements and detection geometries, such as the use of confocal optics, which can improve the S/N at low signal levels by effectively reducing the scattered light from the capillary.

3.4 Single-molecule imaging: rhodamine 123

To further apply high-power blue LEDs toward sensitive detection, we used an LED to image individual rhodamine 123 molecules, which is a cell-permeant green fluorescent dye commonly used to assay mitochondrial membrane potential [59, 60]. Figure 4A shows the optical setup and Fig. 4B the image of individual dye molecules that were immobilized on the glass surface of a coverslip. The achieved S/N ratio of 20 was quite good, but the total integration time required to reach this S/N was 15 s. This integration time is suitable for static imaging and for single-mol-

ecule detection, but is too slow for most dynamic studies at the single-molecule level. At fast integration times (100 ms), which required the use of an Ar^+ laser, the imaged molecules in Fig. 4B exhibited blinking (*i.e.*, a sudden drop in emission as the molecules are trapped in the nonemissive triplet state before returning to the absorption and emission cycle), which further confirmed they were single molecules.

3.5 Single-molecule imaging: λ -DNA molecules

Detection of individual or fragments of individual DNA molecules has proven to be a rapid and sensitive method to identify bacterial DNAs and in DNA fingerprinting. For example, using single-molecule fluorescence detection with an Ar^+/Kr^+ laser operating at ~ 20 mW (488 nm) and a capillary-based flow cytometer, Larson *et al.* [61] successfully demonstrated that different strains of Gram-negative *E. coli* and six different strains of Gram-positive *Staphylococcus aureus* were distinguishable by analyzing the restriction fragments with DNA sizing. With the availability of intercalating DNA dyes, the length of individual DNA molecules can also be determined by measuring the total fluorescence signal, as the amount of the intercalated dye is proportional to the length of the DNA fragment [11].

Figure 5 shows a time series of fluorescence images of single λ -DNA molecules under slow flow (~ 20 $\mu\text{m/s}$) acquired using a blue LED as the excitation source. The

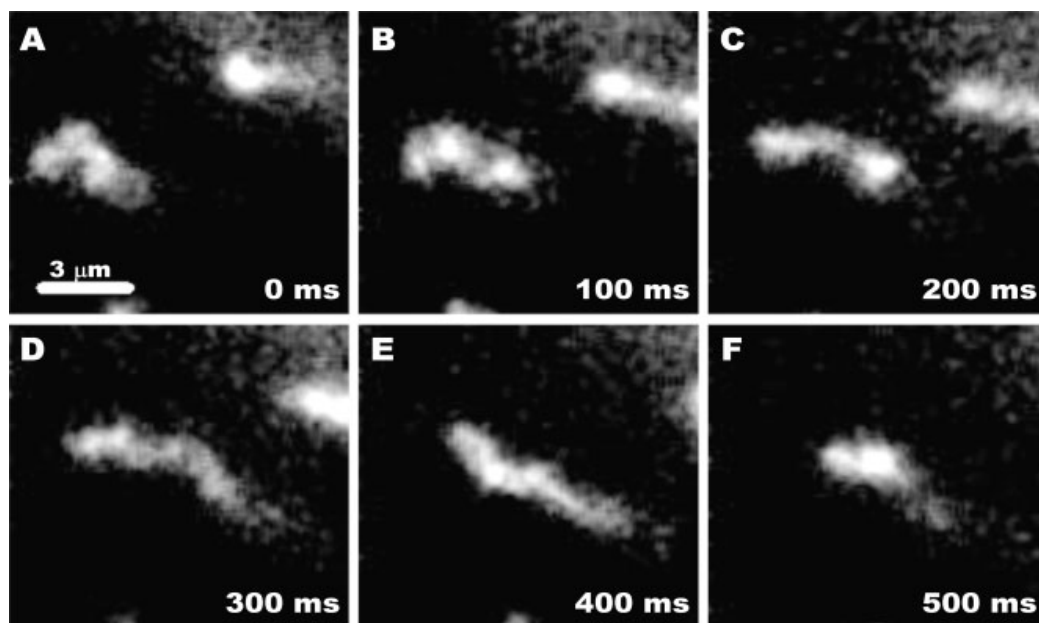


Figure 5. (A)–(F) Sequential images of a single λ -DNA molecule (~ 48 kbp) in a PDMS microchannel (200 μm in width) under gravity-driven slow flow (~ 20 $\mu\text{m/s}$). DNA was intercalated with YOYO-1 at a ratio of ~ 0.6 YOYO:basepair of DNA. Images were collected with 100 ms integration times.

S/N here was excellent, because the sensitivity requirement for imaging single DNA molecules was not as demanding as that for single dye molecules, owing to intercalation of multiple fluorophores (YOYO-1) into a single DNA. The blurry edges of the molecule were caused by the rapid contraction and extension of the DNA in solution, which was faster than our image integration time (100 ms). In this random-coil form, the length of the λ -DNA measured 5–7 μm , but these molecules can extend up to 15–20 μm under shear flow conditions [27].

4 Concluding remarks

We have found that high-power UV/blue LEDs are cost-effective excitation sources in fluorescence sensing. Miniature and with high-emission power, blue LEDs are excellent alternatives to the 488 nm line of an Ar^+ laser in applications that do not require spatial coherence and monochromaticity of the light emission, which is the case for most point detection in separation and microfluidics. In addition to exciting fluorescence, the high emission power of the UV/blue LEDs should benefit other methods of optical detection commonly used in analytical separation, such as absorbance. In our laboratory, several high-power blue/UV LEDs have been in service for more than one year at an estimated average daily usage of one to two hours, with no noticeable degradation in performance. Incidents of early failure have all been traced to poor heat dissipation (e.g., improper encapsulant) or failure of wire bonding. Requiring neither complex cooling nor a large footprint on the optical table, we believe high-power LEDs are a promising excitation source for portable integrated analytical devices, especially in chip-based applications.

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