Cost-Effective Teacher

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# Using a Diode Laser for Laser-Induced Fluorescence

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Diode lasers have become ubiquitous and are used in electronic equipment ranging from laser pointers to compact disc players. These remarkable semiconductor devices operate on the principle that an applied voltage excites electrons above the band gap, and their relaxation results in the emission of monochromatic light whose wavelength is determined by the band gap, which mainly depends on the particular semiconducting material used and how it is doped. Various other details related to the production of coherent radiation are involved, and an excellent review has been published in this *Journal* (*1*).

These lasers operate on low voltages and currents, are durable, and may be purchased for as little as twenty dollars, even in single quantity. They have many properties in common with more sophisticated and expensive lasers, and most contain an internal photodiode that can be used to stabilize and monitor their optical power. The development of diode lasers operating at various wavelengths is a very active field of research. Devices emitting light in the wavelength range of 635–1600 nm are commercially available, and blue diode lasers emitting radiation as short as 400 nm are in the process of being commercialized (*2*).

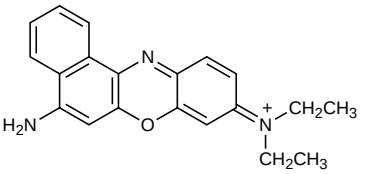
Unlike UV–vis absorbance spectroscopy, fluorescence is a zero-background method and is extremely sensitive, assuming that the analyte of interest fluoresces. The principle of fluorescence spectroscopy is that light incident on a sample causes electronic transitions in an atom or molecule, and the subsequent decay results in the emission of light of a longer wavelength than the excitation radiation. The emitted light is generally proportional to the concentration of the fluorescing analyte. The method is typically carried out by measuring emission perpendicular to the direction of the excitation light, thereby minimizing the detection of scattered excitation radiation. Removal of excitation light in the emission path may be performed by optical filters or a monochromator. In a commercial fluorimeter, a relatively expensive photomultiplier tube (PMT) and gas discharge lamp are used as detector and light source, respectively.

When a laser is used as the excitation source, the technique is termed "laser-induced fluorescence" and offers advantages of improved sensitivity and selectivity. If the excitation and emission spectra are known, a laser may be chosen at a wavelength appropriate to excite fluorescence, and a low-cost photodiode may be used to measure fluorescence. The less efficient detector, relative to a PMT, may be utilized since the excitation portion of the experiment is being performed more efficiently compared to the use of a broadband light source, such as a gas discharge lamp.

While laser-induced fluorescence using a red helium–neon laser has been demonstrated (*3*), diode lasers offer an even more economical approach. These have been used as excitation sources for the ultrasensitive detection of labeled reagents (*4*) and for measurement of fluorescent species by capillary zone electrophoresis (*5*) and chromatography (*6*). The subject of this paper is the assembly of a relatively low-cost diode laser fluorimeter and its use in the teaching laboratory to measure the concentration of a dye dissolved in methanol. Furthermore, this homemade instrument can be used to monitor the oxidative decomposition of the dye by sodium hypochlorite, the active ingredient in laundry bleach.

## Experimental Procedures

The dye studied in this work is Nile blue A (Sigma-Aldrich); its chemical structure is shown below.




Nile blue A

Figure 1 shows the absorbance spectrum of a 1-ppm (part per million, by weight) solution of this dye dissolved in methanol. A variety of diode lasers emitting different wavelengths are commercially available, and one should be cho-

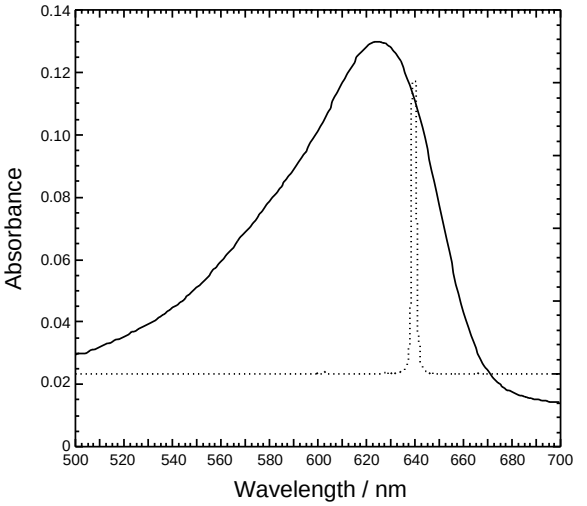



Figure 1. Visible absorbance spectrum of 1 ppm ($1.1 \times 10^{-6}$ M) Nile blue A dissolved in methanol (solid curve) and the emission of the nominally 635-nm diode laser (dashed curve). The absorbance spectrum was acquired on a Perkin Elmer Lambda 9 spectrometer scanning at a rate of 6 nm/min with a 2.00-nm slit, and the laser emission was acquired with an Ocean Optics CHEM2000 spectrometer.

sen that approximately matches the absorbance maximum of the dye. A nominally 635 nm diode laser (Sanyo DL-3038-051) was chosen for this study, and a scan of its emission is also shown in Figure 1. As seen in the figure, the output of the diode laser matches the absorbance spectrum of the dye.

Several versions of the diode laser fluorimeter with different degrees of sophistication have been constructed. The following components are required: a diode laser, a power regulation circuit to drive the laser, two lenses, a long-pass optical filter to remove scattered excitation light and a photodiode detector and amplification circuit. A schematic of the instrument is provided in Figure 2. The lenses and long-pass filter may be purchased inexpensively from commercial vendors such as Edmund Scientific. These may be individually mounted with "five-minute"-type epoxy to small bases that can be easily repositioned to align the optics. Household items such as children's building blocks can be used or small custom-made bases can be constructed out of plastic, aluminum, or wood. Using this philosophy, we have fabricated homemade versions of this fluorimeter, complete with all electronics and optics, for approximately $250. However, if cost is not an issue, it is more convenient to use commercial optic mounts from vendors such as ThorLabs, Inc. In our fluorimeter, a cuvette holder was modified from a scrapped absorbance spectrometer.

Referring to Figure 2, a 25-mm diam, 25-mm focal length double convex lens was used to focus the light from the diode laser into the center of the cuvette. A second, similar lens was placed at an appropriate distance between the cuvette and the detector so that the fluorescence was imaged onto the active portion of the photodiode. The optical filter was a 1-inch RG665 long-pass filter (Edmund Scientific #J45-070). This filter served to absorb scattered excitation light and transmit fluorescence, which for Nile blue A is peaked at approximately 672 nm (*4*). Without this filter, the detector would be swamped by scattered laser light.

To aid in aligning the optics, dilute milk may be placed in the cuvette. The milk scatters the laser light and allows it to be easily seen on a white business card in a darkened room. With the long-pass filter removed, the positions of the lenses and detector can be adjusted so that the scattered light falls on the photodiode. The optical filter can then be inserted in the emission path. Of course, the entire instrument needs to be covered in a light-tight box during the fluorescence measurements to eliminate room light.

As mentioned previously, diode lasers contain an internal photodiode that may be used to regulate the optical output. To take advantage of it, a feedback driver circuit is needed that varies the current to maintain an optical power ("automatic power control"). Various options exist. One is to purchase a battery-powered driver circuit that can be soldered directly to the laser. These are commercially available from vendors such as Meredith Instruments (e.g., part no. LDD-15) or ThorLabs (part no. LD1100) for as little as $15. Alternatively, more costly (< $1,000) diode laser controllers can be purchased from vendors such as ThorLabs or Wavelength Electronics. The controllers are more versatile and have digital readouts of forward voltage, forward current, and optical power. We have tested both options for use in the fluorimeter and found them to perform comparably. The data shown in this paper were acquired using a Wavelength Electronics (LFI4505) diode laser controller.

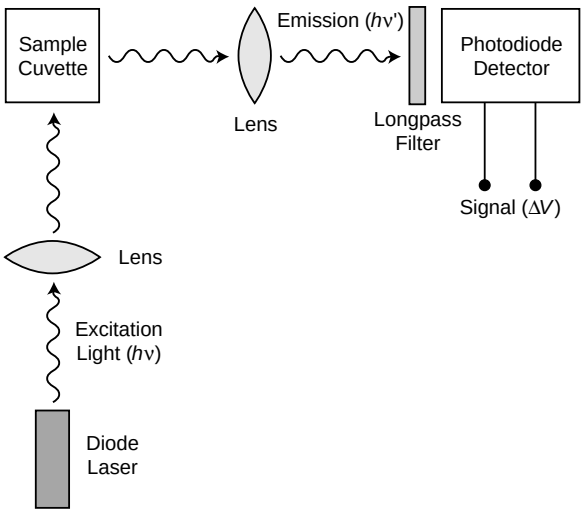



Figure 2. Schematic of the diode laser fluorimeter used in this work. The output of the 635-nm diode laser was focused into the middle of the sample cuvette by a 25-mm focal length double convex lens. The fluorescence was focused by a similar lens onto the active area of the photodiode detector, with the long-pass RG665 filter inserted in the emission path to remove any scattered laser light. The signal from the detector was read by a digital voltmeter.

A variety of simple circuits exist for constructing high-gain photodiodes (*7*), and their use as a fluorescence detector has been demonstrated (*8*). The homemade detector used in the present study was a 31.0-mm$^2$ silicon photodiode (Melles-Griot part no. 13DSI009) whose output was fed to a homemade current-to-voltage amplification circuit consisting of a field-effect transistor (FET) operational amplifier (Burr Brown OPA627) with a 100 MΩ feedback resistor. The detection circuit was mounted in a small aluminum box with a coaxial "BNC"-type connector. It was important to electrically ground the aluminum box to reduce noise pickup. The voltage output of the detector circuit was proportional to the amount of light impinging on the photodiode (and hence to the fluorescence) and could be read by a digital voltmeter or fed into an analog-to-digital converter and sent to a computer. It should be noted that there is nothing particularly special about this detection circuit, and any amplified photodiode detector may be used.

## Hazards

While diode lasers typically have an optical output of only a few milliwatts, shining the light from any laser directly into the eye should be avoided.

## Results and Discussion

Figure 3 shows a plot of fluorescence signal versus Nile blue A concentration for the dye dissolved in methanol at concentrations of 1 ppm (1000 ppb) and less. This is the linear range of the fluorescence–concentration curve, but higher concentrations were observed to give a nonlinear response. For example, the signal for a 2000 ppb solution was 455 mV. Leveling off of fluorescence at higher concentrations is most likely due to self-absorption of fluorescence by the dye itself.

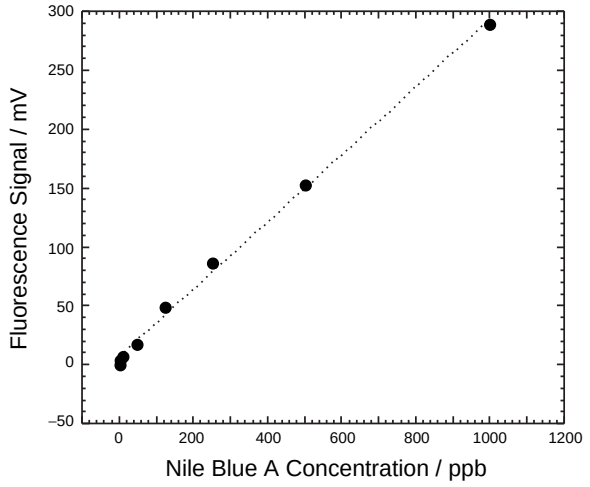



Figure 3. Fluorescence signal versus concentration for Nile blue A dissolved in methanol. The diode laser was operated at 41 mA and 2.20 V forward voltage. A linear fit through the data points is shown, and the slope of this fit is 0.287 mV/ppb.

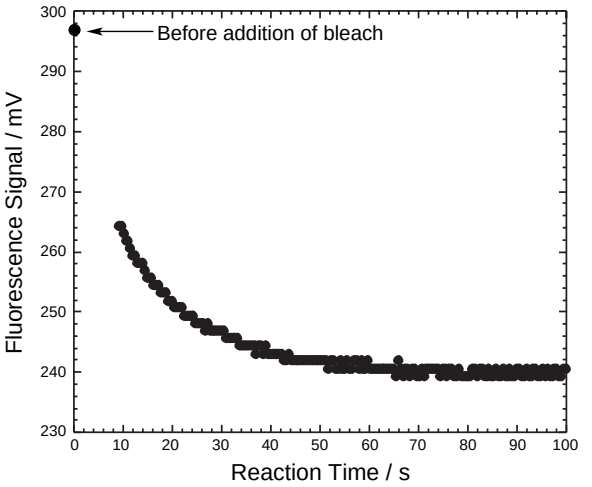



Figure 4. Fluorescence signal for 1 ppm Nile blue A in methanol versus time after addition of diluted household bleach. The point at zero time represents the dye signal prior to addition of bleach. Approximately 9 seconds elapsed between the start of the reaction and when the cuvette was placed in the fluorimeter.

The excellent sensitivity of the diode laser fluorimeter is evidenced by the detection of dye at concentrations as low as 1 ppb.

Dyes such as Nile blue A are susceptible to oxidative degradation by strong oxidants such as sodium hypochlorite, the active ingredient in household bleach. An interesting article in this *Journal* (*9*) reported monitoring the oxidation of bromocresol green with a diode laser, but not by fluorescence. The authors measured the decomposition by shining a diode laser through the dye solution and monitoring absorbance changes. Our homemade fluorimeter provides a complementary and more sensitive approach for studying the oxidative decomposition of dyes.

Toward this end, the following experiment was performed. The output of the photodiode detector was monitored by a Macintosh computer using a serial interface box (Vernier Software). A sample of household bleach (Clorox brand) that contained 5.25% sodium hypochlorite was diluted by a factor of 1000 (by weight) in methanol. Using a micropipet, 100 µL of this dilute bleach solution was added to 3 mL of a 1 ppm solution of Nile blue A in methanol that had been placed in the cuvette. Immediately upon addition of bleach, the cuvette was covered with a piece of Parafilm and shaken. It was then quickly placed in the fluorimeter, and the fluorescence signal was collected by the computer. The results are shown in Figure 4. The data clearly indicate oxidative decomposition of Nile blue A by sodium hypochlorite.

The activity of the 1:1000 methanol-diluted bleach solution was found to decrease with time, and this led to irreproducible results with respect to the volume of bleach solution needed to yield the same rate of decay. Detailed kinetics investigations were not performed in this study, but these certainly should be possible if the bleach is diluted and added consistently to the dye solution. For the experiment shown in Figure 4, the 1:1000 bleach solution sat around for approximately one hour before being used.

This paper describes the construction and use of a sensitive diode laser fluorimeter. The use of red lasers as excitation sources limits the analytes that can be detected to ones having low-lying excited states, but blue diode lasers are now being commercialized (*2*) and will extend the range of this type of instrument. An important feature of the described fluorimeter is that its components are transparent to the students. While laser-based fluorimeters are used in chemical research because of their higher sensitivity, it should be pointed out to the students that commercial fluorimeters do not usually contain a laser, but instead have a broadband light source whose output is monochromatized by a diffraction grating. Instead of a long-pass filter, a grating is often used to prevent scattered excitation light from reaching the detector.

## Acknowledgment

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## Literature Cited

1. Baumann, M. G. D.; Wright, J. C.; Ellis, A. B.; Kuech, T.; Lisensky, G. C. *J. Chem. Educ*. **1992,** *69*, 89–95. Condren, S. M.; Lisensky, G. C.; Ellis, A. B.; Nordell, K. J.; Kuech, T. F.; Stockman, S. A. *J. Chem. Educ.* **2001,** *78*, 1033–1040.
2. Gunshor, R. L.; Nurmikko, A. V. *Sci. Am*. **1996,** *275* (1), 48–51.
3. Zare, R. N.; Spencer, B. H.; Springer, D. S.; Jacobson, M. P. *Laser Experiments for Beginners*; University Science Books: Sausalito, CA, 1995.
4. Imasaka, T.; Tsukamoto, A.; Ishibashi, N. *Anal. Chem*. **1989,** *61*, 2285–2288.
5. Higashijima, T; Fuchigami, T.; Imasaka, T.; Ishibashi, N. *Anal. Chem*. **1992,** *64*, 711–714.
6. Mank, A. J. G.; Molenaar, E. J.; Lingeman, H.; Gooijer, C.; Brinkman, U. A. Th.; Velthorst, N. H. *Anal. Chem*. **1993,** *65*, 2197–2203.
7. Graeme, J. *Photodiode Amplifiers: Op Amp Solutions*; McGraw-Hill: New York, 1996.
8. Whitten, J. E.; Alfano, J. C. *J. Pulp Paper Sci*. **1999,** *25* (1), 21–24.
9. Zimmer, J.; Reeves, J.; Jones, R.; Cizerle, K.; Ward, D. *J. Chem. Educ*. **1995,** *72*, A115–A120.