
Introduction to Fluorescence in General Chemistry Using the Intercalation of Propidium Iodide with DNA

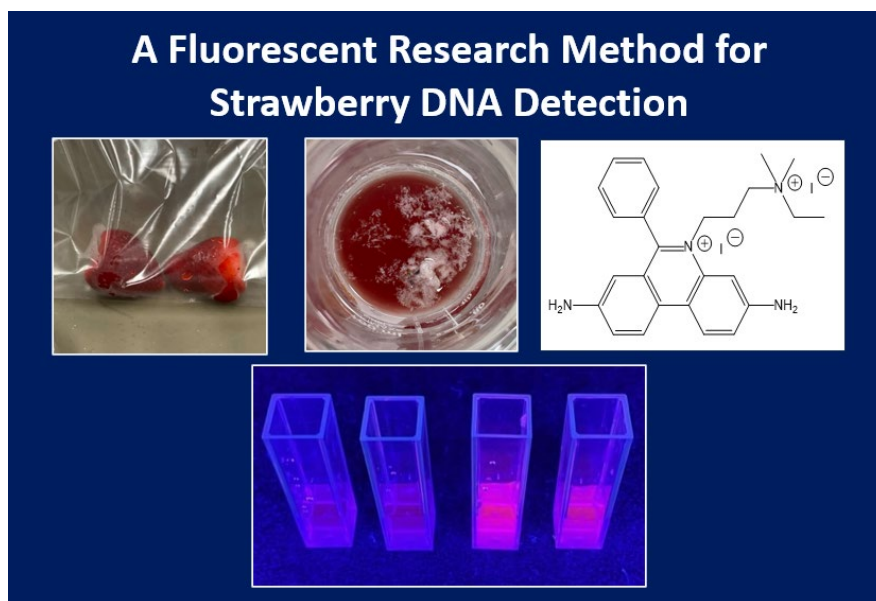
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ABSTRACT

The phenomenon of fluorescence is very important from multiple standpoints in the chemical and biological sciences. This paper introduces an experiment in a first-semester chemistry laboratory course that uses a current biomedical research method, the detection of double-stranded DNA using the intercalator propidium iodide. Fluorescence is detected both using blacklight illumination and also with white light and a spectrometer, using the two excitation bands for propidium. This experiment also involves students obtaining DNA from strawberries and then determining the amount of DNA they have isolated using fluorescence methods. The experiment provides students with an initial experience in fluorescence-based analytical chemistry and the concepts of fluorescence as a quantum phenomenon.

GRAPHICAL ABSTRACT



KEYWORDS

Laboratory, fluorescence, imaging, DNA, quantum mechanics, analytical chemistry.

INTRODUCTION

Fluorescence is one of the most important methods in contemporary analytical chemistry, especially in biochemical settings. This is apparent in multiple contexts, including the presence of fluorescence as a key method behind three different chemistry Nobel Prizes,^{1–3} the widespread use of fluorescence as a tool for analytical and kinetics experiments, and the presence of fluorescent systems in popular settings, not least as the basis of the “dyeing” of the Chicago River in celebration of St. Patrick’s Day (Figure 1).⁴ Fluorescence is also an important tool in biomedical settings, for example in the use of indocyanine green (ICG) in arthroscopic and robotic surgery.⁵ Because fluorescence involves multiple molecular energy levels (the ground state, first excited state, and the intermediate fluorescent state), it also provides a direct way to encounter fundamental quantum concepts.



Figure 1. Dyeing of the Chicago River with an orange powder to yield a bright green color.

There have been several experiments developed for educational settings involving fluorescence reported in this journal. Many of these occur in upper-level courses with the few that exist for general chemistry settings using fluorescence intensity as a source of data for another chemistry content area.

For example, Jain and coworkers have reported on an equilibrium experiment involving the molecule proflavine, which has its fluorescence quenched when bound to DNA.⁶ Matsuoka and Akitsu reported a study of the kinetics of the hydrolysis of t-butyl chloride using the difference between the fluorescent and non-fluorescent forms of fluorescein as an indicator of the related pH change.⁷ A few papers discuss the relationship of fluorescence to concepts of quantum mechanics and molecular energy. Miao and Thomas reported an experiment in an upper-division biochemistry lab where students do a context-based group activity to support understanding of myoglobin structure and denaturation and also engage concepts of energetics, quantum yield, and different decay processes.⁸ Convenient measurement of fluorescence is also important in several published studies, instead of an expensive fluorimeter. In one case, Koenig *et al.* discussed how an SLR camera can be used as the detector with an iPad screen as an excitation source and an ImageJ/ MATLAB program for analyzing the digital images.⁹ In another, Lu and coworkers examined a smartphone is used to examine different concentrations of rhodamine-B and also its reaction with hypochlorite, accompanied by the use of *ImageJ* as software to determine relative fluorescence intensities.¹⁰

Many context-based experiments exist as well, where fluorescence is used with common materials. There are examples of where students engage in applications of fluorescence in cultural ways, as in the report by Jefferies *et al.* of how different promoters are used in a biochemistry course for “Agar art.”¹¹ Fluorescence is also useful as a simple analytical methods, as Smith and coworkers have reported an upper-division experiment where fluorescence is used to determine caffeine concentration by its effect on fluorescence of aspirin.¹² Selective binding of fluorophores to biomolecules is a major component of contemporary biochemistry. This, for example, was the major application that Carolyn Bertozzi and her coworkers developed using click chemistry, exemplified by “CalFluor” molecules.¹³ This has also been incorporated in instruction by Fura *et al.* in an experiment on bio-orthogonal chemistry.¹⁴

At our institution, we use two fluorescence experiments in second semester general chemistry: the equilibrium experiment by Jain and coworkers⁶ involving quenching of the intercalating proflavine when bound to DNA, and our own coumarin-102 kinetics experiment. We were also interested in developing a laboratory experiment that would serve to introduce students to fluorescence in a first-semester lab that would also engage them with a biological molecule (DNA) as an analyte. In this case, we examine

the binding of propidium iodide (Figure 2) to DNA. It can be used as a method for imaging DNA in cells after they have died and their cell membrane is disrupted. As a result, propidium iodide can be used to determine if a cell is dead and it is used, in conjunction with counterstains that detect living cells as the basis of “live-dead” cell assays.¹⁵ The strong binding of propidium iodide to DNA is similar to that of the related intercalator ethidium bromide,¹⁶ However, propidium iodide has no reported toxicity concerns, possibly because it is dicationic and will not penetrate intact cell membranes.¹⁷

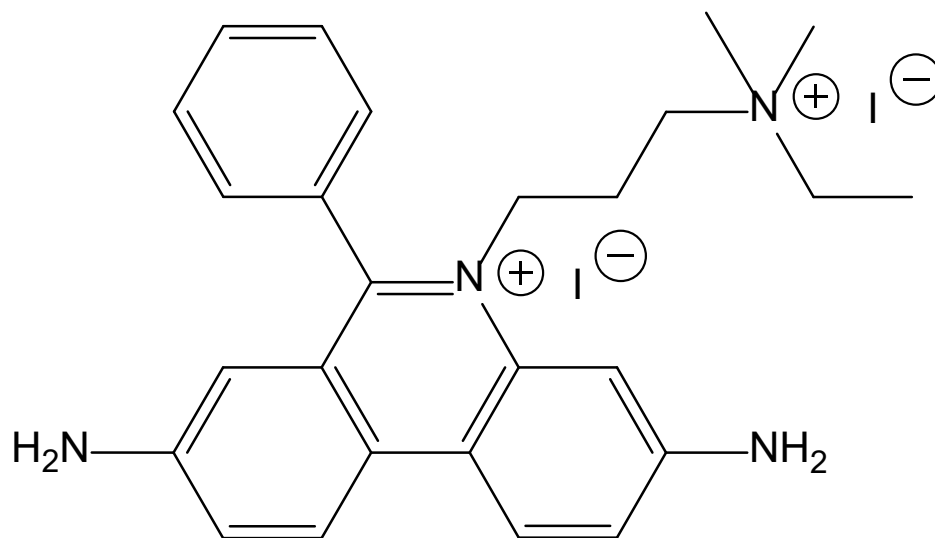


Figure 2. Structure of propidium iodide

When propidium iodide is bound to double-stranded DNA, it exhibits strong fluorescence in the red if it is excited with either near-UV or blue-green light (Figure 3).

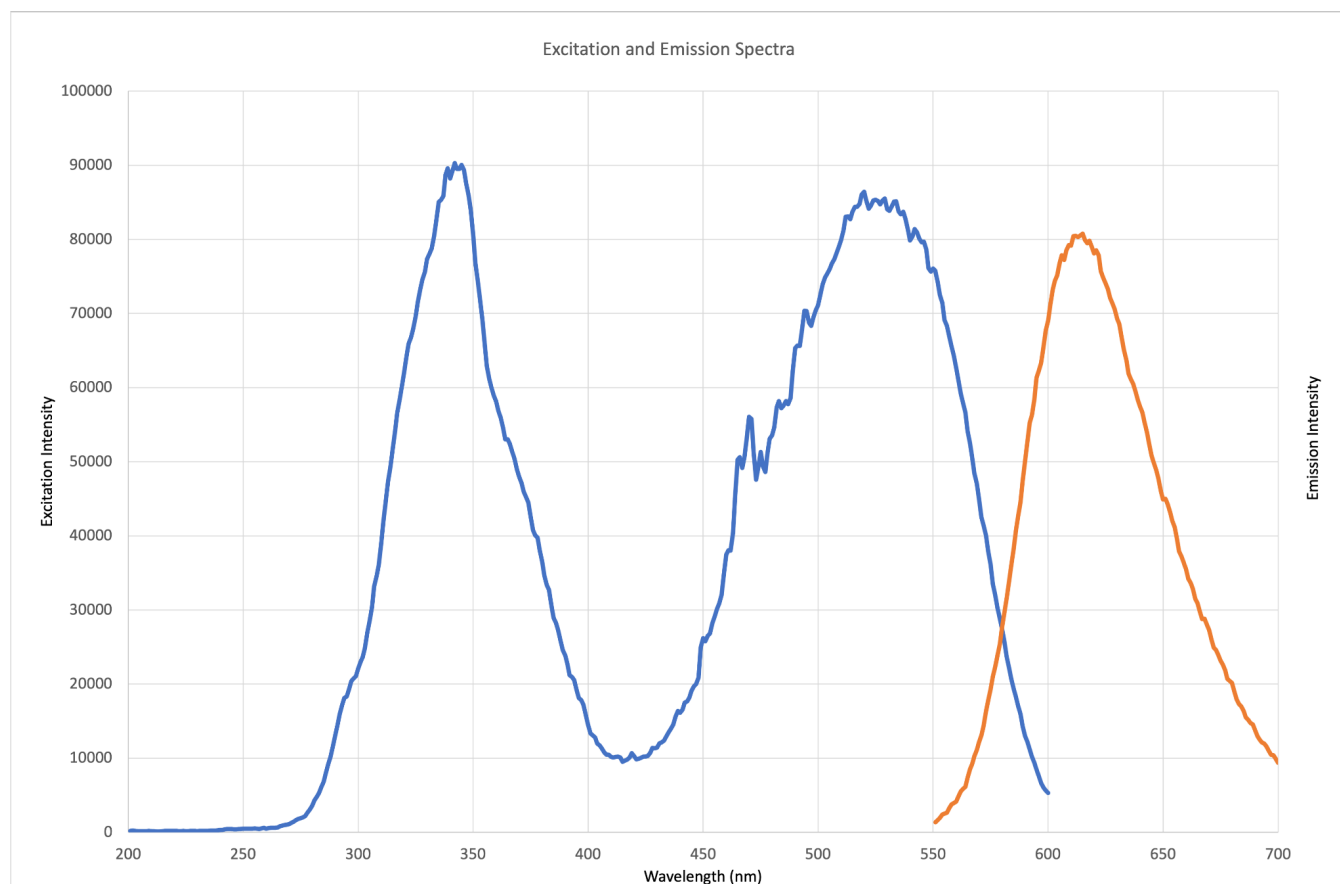


Figure 3. Excitation and emission spectrum for propidium iodide in the presence of DNA

In this procedure, we also make use of a widely used method for isolating DNA from strawberries. This simple procedure yields a white gelatinous material that, in most cases, is simply declared to be DNA.¹⁸ But, as Kocheril *et al.* have reported, it is possible to demonstrate that the material is actually DNA through Raman spectroscopy.¹⁹ The use of a DNA binding fluorophore allows a similar proof to be done in a simpler way appropriate for an introductory lab course.

Experimental Procedure

The experimental procedure was developed in the context of a structured research program, the STEM Initiative CoLab, that provides students with a workshop-based research experience. We have previously described how the CoLab program works with entering college students as a way to engage in open-ended work that also supports learning nature of science and communication skills.^{20,21} In the summer 2017 implementation of CoLab, students were given the opportunity to explore multiple aspects

of the binding of propidium iodide to DNA, to cells that had been killed, and to material isolated from strawberries. These preliminary results gave us guidance for more direct development of practical methods to introduce the binding of propidium iodide to DNA for the present work.

In the present laboratory activity, students were directed to produce five DNA standards with buffer using a DNA stock solution. These were done directly in 1cm plastic cuvettes into which a measured volume of propidium iodide was added. These standards were illuminated with a commercially available LED UV blacklight bar and then photographed using a cellphone camera (Figure 4).

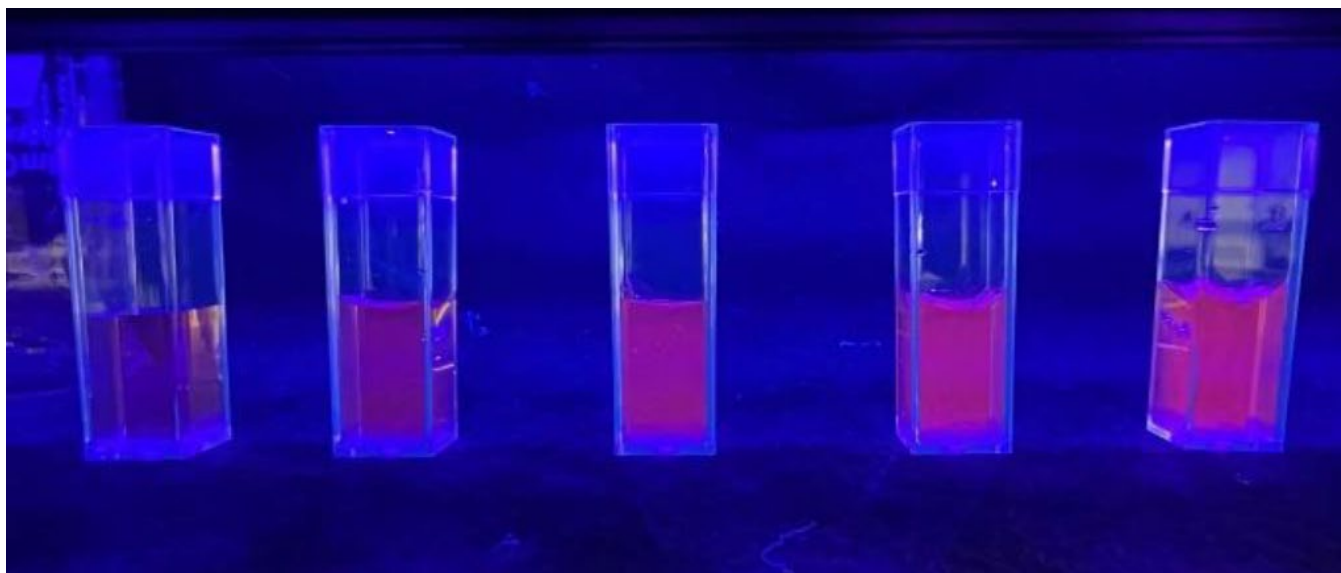


Figure 4. Samples of propidium iodide in the presence of varying amounts of DNA under blacklight illumination (Student FA22_136)

Students are shown how to analyze the photo using *ImageJ* but, to reinforce the connection to conventional optical spectroscopy, quantitative fluorescence intensity data is also obtained using an Ocean Optics Red Tide USB650UV or Ocean Insight Flame spectrometers, with samples illuminated from above with a white led lamp (Figure S-3). Students record the fluorescence intensity for the standard solution at wavelengths in the range of 610-630 nm. The plotted graph shows a direct correlation between standard solutions with different concentration of DNA and the fluorescence intensity. The linear regression analysis is then used by students to find the DNA concentration.

Hazards

Part one of the experiment uses materials that are commonly found in homes and the procedure is often used in outreach settings. This includes isopropyl alcohol, which is flammable. The phosphate buffer and DNA solutions present minimal hazards, since the pH is at an intermediate value and the DNA concentrations are low. Eye protection is still essential in this setting. Propidium iodide, as noted, is an intercalator and a biologically active material, given its use in assays of cell survival.¹⁵ There are no specific reports of hazards associated with propidium iodide, even in an assay that documented risks of other related dyes such as ethidium bromide.¹⁷ Nevertheless, students are directed to be very careful with these solutions, including wearing appropriate gloves.

IMPLEMENTATION AND STUDENT RESULTS

This lab was introduced into a general chemistry laboratory course at a large midwestern urban university in the USA. The laboratory is a two-credit, one-semester course taught independently of the introductory lecture course and includes 12 labs over a 15-week semester. Labs meet weekly with up to 24 students and one graduate teaching assistant (TA) for three hours. The course serves students in traditional chemistry and biochemistry tracks, as well as other academic majors such as biology and physics, and preprofessional tracks—including nursing, pre-pharmacy, and pre-medicine.

Prior to coming to lab, students watched a 20-minute video lecture by the second author covering binding of biological molecules, examples of fluorescence in science and their world, and quantum mechanics as it applies to fluorescence (see supporting information). The lecture also outlines the objectives and procedures of the lab activity. A first-person video of the lab procedure and a video on how to complete the post lab are also made available to students on the university's learning management system (LMS). Students are required to write a summary of the lab procedures from the manual and take a pre-lab quiz that covers concepts and basic procedures from the lecture and introduction before entering lab. In lab, TAs present a short lecture on safety and waste disposal and then students pair up to complete the lab.

The lab activity is divided into three parts. In the first part, students extract DNA from fresh or frozen strawberries following the procedure described by Gahlon¹⁸ in which deionized water, salt, and

non-fluorescent dish soap are mixed and then agitated in a resealable plastic bag. The mixture is allowed to settle and the solution at the top is decanted into a beaker through a small gap in the zipper. We have found that, if this is done carefully, it is not necessary to carry out a time-consuming filtration. TAs are instructed to examine students' filtrates and suggest mechanical removal of solids with tweezers or allow the solution to resettle and decant again. Cold isopropyl alcohol is layered above the decanted strawberry solution which is left in an ice bath for approximately 30-45 minutes, while part two is done. The DNA that forms at the interface between the alcohol and solution is extracted, dried, weighed, and set aside for part three (Figure 5).



Figure 5. Isolation of DNA from strawberries

In part two students prepare μM standard solutions of DNA with propidium iodide and buffer solutions (see table 1). Students use Ocean Optics spectrometers in fluorescence mode to collect intensity data and produce a calibration curve based on spectrometer values. Finally, in part three students dissolve their DNA in 50. mL of buffer, add propidium iodide to the solution, and put it into cuvettes. Students repeat the same procedure with the blacklight and the fluorescence spectrophotometer as they did with the standards. They use the spectrophotometer data and the calibration curves to determine the μM concentration of the DNA in their samples and an estimate of the percentage of DNA in the strawberry.

Typical data from a consented student are shown in Figure 6 and Tables 1 and 2. We have also examined the general trend of student results by using a sample of the reports from two semesters.

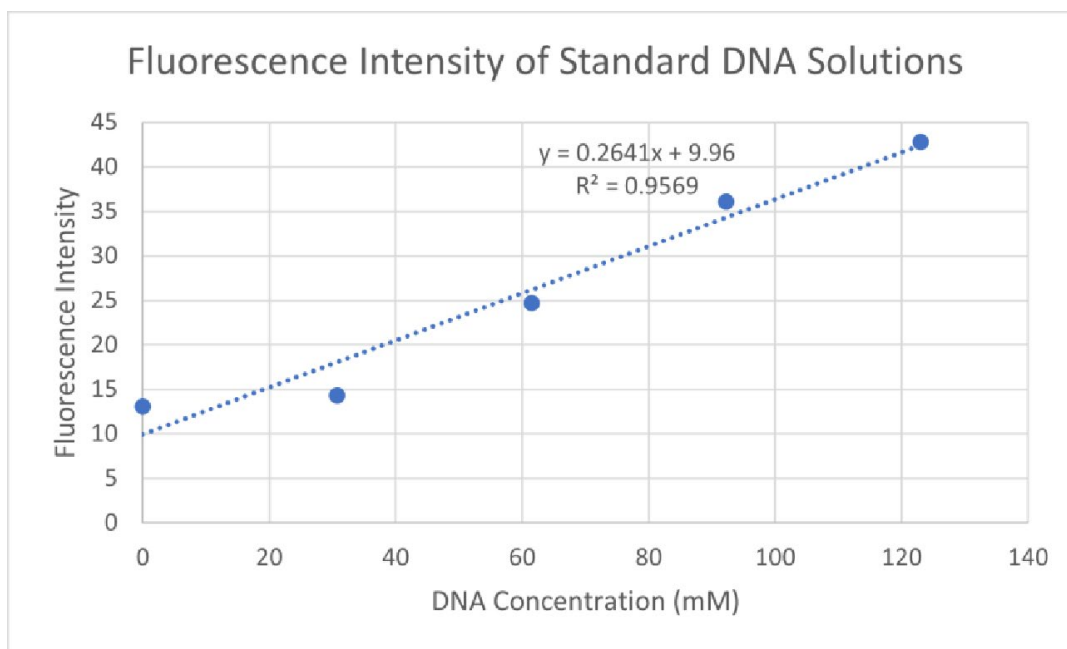


Figure 6. Spectrometer data for fluorescence of propidium iodide with DNA.
(Student FA22_136)

Table 1. Sample Standardized Fluorescence Measurements for Propidium Iodide with DNA (Student FA22_136)

	Propidium Iodide (mL)	DNA (μ L)	Buffer (μ L)	Concentration of DNA (μ M base pair)	Fluorescence intensity (count)
A	1.50	0	500.	0	13.1
B	1.50	100.	400.	30.8	14.3
C	1.50	200.	300.	61.5	24.7
D	1.50	300.	200.	92.3	36.1
E	1.50	400.	100.	123	42.8

Table 2. Sample student Data on Determination of DNA from Strawberries
(Student FA22_136)

	Propidium Iodide (mL)	DNA (μ L)	Buffer (μ L)	Fluorescence intensity (count)	Concentration of DNA (μ M base pair)
Strawberry Sample 1	1.50	500.	0.00	18.9	33.8
Strawberry Sample 2	1.50	500.	0.00	14.2	16.0
Strawberry Sample 3	1.50	500.	0.00	20.0	38.0

For assessment of student outcomes, we consented students in the Fall 2022 and Fall 2023 semesters, according to an approved IRB protocol. In Fall 2022, 266 of *ca.* 600 students gave permission to use their lab reports for this study. In Fall 2023, 264 out of *ca.* 680 students did so. Out of these, we randomly selected 40 Fall 2022 reports and 20 Fall 2023 reports for analysis. We analyzed the lab reports for overall performance and for characteristics of understanding fluorescence and its applications.

In Fall 2022, 37 of the 40 reports analyzed reported a DNA calibration curve with an average R^2 of 0.94; 27 of the reports had R^2 values at or above 0.90. In Fall of 2023 we modified the instructions for the TAs and the students on positioning the illumination light LED and in that case, we found 80% of the reports had R^2 values at or above 0.90. Concentrations of DNA solutions from recovered strawberry DNA were reported by only twenty-three students in Fall 2022 with an average of 10. mg DNA per gram of strawberry. Of those who did not report DNA amounts, nine had an error in their calculations but had good data and seven had a negative concentration for their DNA because of a problem measuring the fluorescence intensity. In Fall of 2023 successful determination of DNA from the strawberries was done by 16 of the 20 students in the data sample.

We also probed student learning of relevant principles of fluorescence. We included short answer questions to assess student understandings. Almost all students inferred the relationship between fluorescence intensity and DNA concentration appropriately. A typical answer to the prompt “Provide an analysis of your photograph of fluorescent solutions under blacklight illumination” in the postlab was “The higher the concentration of DNA, the brighter the fluorescence. All the way on the right (cuvette E) there was the highest concentration of DNA (400) and on the left was the least amount of DNA (0)” (FA22_018). The second question in the postlab was “Provide an analysis of restrictions on the method.” Regarding the limitations discussed in the question, over one third of the students identified numerous human errors during the preparation process. For example, one student wrote, “Some restrictions of this method is [*sic*] that it relies on the solutions inside the cuvettes to be carefully made and if the solutions are not correctly made, the calculations of the unknown DNA sample solution can be incorrect” (FA22_074).

In Fall 2023 we also asked students to connect the experience of this lab to their “real life” through a question on their favorite “glow in the dark” experience. Most students mentioned experiences in social settings, such as a laser tag or other party experience. But some mentioned glowsticks and some discussed the use of paints that emit light in the dark. In the future, we will include a clearer discussion of how these chemiluminescent phenomena are different from fluorescence.

Overall, we met all objectives in this experiment. Students successfully extracted DNA from strawberries. They used fluorescence spectroscopy to detect DNA concentration through the fluorescence of propidium iodide. In addition, almost all students identified the relationship of intensity to concentration using linear regression. Most were also able to detect the amount of DNA extracted from sample of strawberries. Future work will examine how the learning in this setting is related to other fluorescence-dependent experiments that are used in later semesters to study kinetics and equilibrium.

ASSOCIATED CONTENT

Supporting Information

Supporting Information is available at _____.

File containing the directions for instructors and students for the activity and slides to present the activity and principles of fluorescence (PDF; DOCX).

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Notes

The authors declare no competing financial interest.

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REFERENCES

- (1) Ehrenberg, M.; Lidin, Sven. *The Nobel Prize in 2014: How the optical microscope became a nanoscope*. <https://www.nobelprize.org/uploads/2018/06/popular-chemistryprize2014.pdf>.
- (2) Fernholm, A. *The Nobel Prize in Chemistry: Their functional chemistry works wonder*. <https://www.nobelprize.org/uploads/2022/10/popular-chemistryprize2022.pdf>.
- (3) The Royal Swedish Academy of Sciences. *The Nobel Prize in Chemistry 2008: How the Jellyfish's Green Light Revolutionised Bioscience*. <https://www.nobelprize.org/uploads/2018/06/popular-chemistryprize2008.pdf>.
- (4) Chicago Tribune. *St. Patrick's Day 2023: Chicago River dyed green*. <https://www.chicagotribune.com/news/ct-xpm-1995-03-16-9503160209-story.html>.
- (5) Daskalaki, D.; Aguilera, F.; Patton, K.; Giulianotti, P. C. Fluorescence in Robotic Surgery. *J. Surg. Oncol.* **2015**, 112 (3), 250–256. <https://doi.org/10.1002/jso.23910>.
- (6) Jain, S. S.; LaFratta, C. N.; Medina, A.; Pelse, I. Proflavine–DNA Binding Using a Handheld Fluorescence Spectrometer: A Laboratory for Introductory Chemistry. *J. Chem. Educ.* **2013**, 90 (9), 1215–1217. <https://doi.org/10.1021/ed300481u>.
- (7) Matsuoka, M.; Akitsu, T. Hydrolysis of 2-Chloro-2-Methylpropane—Demonstration Using the Quenching of Fluorescence from Fluorescein. *J. Chem. Educ.* **2021**. <https://doi.org/10.1021/acs.jchemed.0c01255>.
- (8) Miao, Y.; Thomas, C. L. Using Myoglobin Denaturation To Help Biochemistry Students Understand Protein Structure. *J. Chem. Educ.* **2017**, 94 (10), 1498–1501. <https://doi.org/10.1021/acs.jchemed.7b00035>.
- (9) Koenig, M. H.; Yi, E. P.; Sandridge, M. J.; Mathew, A. S.; Demas, J. N. “Open-Box” Approach to Measuring Fluorescence Quenching Using an iPad Screen and Digital SLR Camera. *J. Chem. Educ.* **2015**, 92 (2), 310–316. <https://doi.org/10.1021/ed500373d>.
- (10) Lu, M.; Yang, Z.; Ding, Z.; Liang, T. A Fluorometer-Free Experimental Course for Fluorescence Analysis Based on Smartphone and Image/Data-Processing Software Using a Synthetic Sensor. *J. Chem. Educ.* **2023**, 100 (9), 3564–3569. <https://doi.org/10.1021/acs.jchemed.3c00252>.

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- (11) Jefferies, L. R.; Giordano, A. N.; Hicks, B. W. Agar Art as an Instructional Tool to Teach Inducible Promoters via Fluorescent Protein Expression. *J. Chem. Educ.* **2022**, *99* (12), 4181–4185. <https://doi.org/10.1021/acs.jchemed.2c00004>.
- (12) Smith, J.; Loxley, K.; Sheridan, P.; Hamilton, T. M. Analysis of Caffeine in Beverages Using Aspirin as a Fluorescent Chemosensor. *J. Chem. Educ.* **2016**, *93* (10), 1776–1780. <https://doi.org/10.1021/acs.jchemed.6b00303>.
- (13) Shieh, P.; Dien, V. T.; Beahm, B. J.; Castellano, J. M.; Wyss-Coray, T.; Bertozzi, C. R. CalFluors: A Universal Motif for Fluorogenic Azide Probes across the Visible Spectrum. *J. Am. Chem. Soc.* **2015**, *137* (22), 7145–7151. <https://doi.org/10.1021/jacs.5b02383>.
- (14) Fura, S.; Oluwole, S. A.; Hakim, Q. A.; Sanchez, H.; Toledo, D.; Quintana, A. A.; Agatemor, C. Bioorthogonal Metabolic Labeling Experiments to Introduce Undergraduate Students to Interdisciplinarity at the Interface of Chemistry and Biology. *J. Chem. Educ.* **2023**, *100* (6), 2394–2401. <https://doi.org/10.1021/acs.jchemed.3c00209>.
- (15) Krämer, C. E. M.; Wiechert, W.; Kohlheyer, D. Time-Resolved, Single-Cell Analysis of Induced and Programmed Cell Death via Non-Invasive Propidium Iodide and Counterstain Perfusion. *Sci. Rep.* **2016**, *6* (1), 32104. <https://doi.org/10.1038/srep32104>.
- (16) Bourzac, K. M.; LaVine, L. J.; Rice, M. S. Analysis of DAPI and SYBR Green I as Alternatives to Ethidium Bromide for Nucleic Acid Staining in Agarose Gel Electrophoresis. *J. Chem. Educ.* **2003**, *80* (11), 1292. <https://doi.org/10.1021/ed080p1292>.
- (17) Kohler, M.; Kündig, A.; Reist, H.-W.; Michel, C. Modification of in Vitro Mouse Embryogenesis by X-Rays and Fluorochromes. *Radiat. Environ. Biophys.* **1994**, *33* (4), 341–351. <https://doi.org/10.1007/BF01210455>.
- (18) Gahlon, H. L. A Brief History and Practical Applications in DNA Extraction: Chemical Education. *Chimia* **2020**, *74* (11), 907–907.
- (19) Kocheril, P. A.; Jones, M. M.; Kubicek-Sutherland, J. Z. Raman Scattering Spectroscopy on Strawberry DNA. *J. Chem. Educ.* **2022**, *99* (10), 3546–3552. <https://doi.org/10.1021/acs.jchemed.2c00687>.
- (20) Wierzchowski, A.; Wink, D. J. Categorizing Student Learning about Research, Nature of Science, and Poster Presentation in a Workshop-Based Undergraduate Research Experience. *J. Chem. Educ.* **2023**, *100*, 2873–2883. <https://doi.org/10.1021/acs.jchemed.3c00174>.
- (21) Wierzchowski, A.; Wink, D. J.; Zhang, H.; Kambanis, K.; Rojas Robles, J. O.; Rosenhouse-Dantsker, A. CoLab: A Workshop-Based Undergraduate Research Experience for Entering College Students. *J. Chem. Educ.* **2022**, *99* (12), 4085–4093. <https://doi.org/10.1021/acs.jchemed.1c01290>.