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Open-Source Fluorescence Spectrometer for Noncontact Scientific Research and Education

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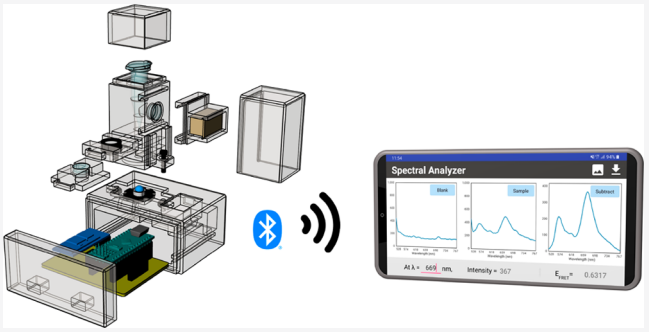
ABSTRACT: Transforming fluorescence spectrometers into cost-effective, portable devices provides the potential for field-based applications in biological, environmental, and clinical research and education. However, the majority of developed spectroscopic technologies continue to require heavy, expensive equipment and trained personnel for operation or do not support multispectral analysis, thereby restricting their use in resource-limited environments. Herein, we report a wireless, portable, cost-effective, open-source fluorescence spectrometer (OpenFS) developed by compactly assembling optical and electronic elements in a 3D-printed housing. OpenFS outputs an accurate emission spectrum over a wide range of wavelengths and demonstrates greater sensitivity for fluorescence quantification compared to a conventional fluorometer. We demonstrate the functionality of OpenFS as a fluorescence resonance energy transfer (FRET)-based DNA sensor by detecting target DNA molecules with FRET efficiency and prove its utility as an Internet of Things device by performing wireless measurements and spectral analysis on a smartphone with a custom-developed Android application. This portable open-source spectrometer can lead to new opportunities in research and educational fields where fluorescence spectroscopy has not been available because of its cost and size and provides the potential for the development of mobile diagnostics platforms.



KEYWORDS: *General Public, Upper-Division Undergraduate, Analytical Chemistry, Biochemistry, Interdisciplinary/Multidisciplinary, Public Understanding/Outreach, Hands-On Learning/Manipulatives, Fluorescence Spectroscopy, Laboratory Equipment/Apparatus*

INTRODUCTION

Fluorescence spectral analysis is extensively used to measure multiple fluorescent compounds in sample solutions in diverse fields ranging from biomedical applications to environmental monitoring.[1−12] Förster resonance energy transfer or fluorescence resonance energy transfer (FRET) requires spectral measurements to quantify the relative intensity between donor and acceptor fluorophores and enables the real-time analysis of molecular structure changes and interactions by accurately measuring molecular proximity.[1−7] Multiple fluorescence colors of quantum dots need to be quantified for multiplexed assays that exploit the unique optical characteristics of fluorescent nanocrystals, such as simultaneous excitation for multiple fluorescence colors and size-tunable emission wavelengths.[7−9] Fluorescence spectroscopic characterization of algal and cyanobacterial cells provides a powerful method to discriminate problematic species and predict algal blooms.[10−12] Fluorescence spectroscopy is a highly versatile and widely used technique for these applications; however, commercial spectrometers remain bulky, delicate, and expensive, which limits their use to well-equipped laboratories.

Miniaturizing spectrometers has a significant potential for field-portable applications and has been addressed by developing small, simple, and cost-effective devices, thereby improving accessibility to the equipment.[13−17] Although not spectrometers, portable fluorometers capable of measuring multiple fluorescence characteristics have been developed for FRET biosensors, the detection of luminescent analytes, and the measurement of quinine in tonic water.[13−15] However, these photodetector-based approaches only provide information on fluorescence intensity without wavelength information and thus have limited applications. Integrating a smartphone and optical grating enables fluorescence spectral analysis by exploiting the embedded imaging sensor and computing power of smartphones and the ability of grating to separate light of different wavelengths with a spatial resolution.[16,17] Smartphone-based spectrometers are promising for mobile healthcare and environmental monitoring as they can potentially integrate Internet of Things (IoT) features into analytical

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equipment; however, Bayer filters of general imaging sensors can cause spectral overlap, which can be a fundamental limit on the spectral resolution.

Recent progress has been made in the development of open-source hardware for frugal science, a growing movement in broad communities that range from professional engineers and scientists to do-it-yourself (DIY) researchers, to make expensive scientific instruments affordable and transportable.[15,18−28] In this context, open-source, DIY spectrophotometers or photometers have been successfully developed;[15,23−27] however, fluorescence spectrometers still remain to be developed. To address the above issues, we present an open-source fluorescence emission spectrometer (OpenFS) that is simple to fabricate without expertise in optics and electronics and provides a spectral sampling interval of approximately 2 nm within a small footprint of 7.3 cm × 6.1 cm × 10.2 cm. At a cost of about $540, this is affordable in resource-limited environments compared to commercial fluorescence spectrophotometers, which cost more than $10,000 (see the Supporting Information, Tables S1 and S2). To demonstrate the capability of OpenFS to perform fluorescence spectral analysis without access to expensive, research-grade equipment, the development of OpenFS and a series of experiments using it were performed by undergraduate students enrolled in an undergraduate research program for biomedical engineering at Hanyang University. We then integrated OpenFS with a FRET-based DNA biosensor to demonstrate its capability for multispectral fluorescence analysis and quantification of a target DNA molecule as part of the undergraduate research program. We also added a wireless communication functionality to OpenFS to extend its applicability to field-portable, wireless spectral analysis by wirelessly transmitting signals to a smartphone via a Bluetooth module and using its computing capability for spectral analysis. OpenFS has great potential as a low-cost alternative to fluorescence spectrophotometers and can be a valuable educational tool for students and nonexpert users to understand the underlying principles of fluorescence emission spectrophotometry. To evaluate the educational utility of OpenFS, we introduced it to undergraduate and graduate students through a training event and received responses from the students with a high level of satisfaction for experimental education of fluorescence spectroscopy and FRET assay using OpenFS.

EXPERIMENTAL SECTION

OpenFS Design and Fabrication

OpenFS was designed to accept a liquid sample between an excitation light source and an optical sensor, similar to the structure of conventional fluorescence spectrometers. It was fabricated by assembling a 3 W blue LED, two aspheric condenser lenses with a focal length of 8 mm (36-164, Edmund Optics), a bandpass filter with a center wavelength (CWL) of 475 nm and full width half maximum (FWHM) of 25 nm (87-776, Edmund Optics), a microelectromechanical systems (MEMS) spectrometer sensor (C12880MA, Hamamatsu Photonics K.K.), a microcontroller board (Arduino Nano), a Bluetooth module (HC-05), and a transistor in a 3D-printed housing that was printed using a 3D printer, Asiga Max UV (Asiga). We used the bandpass filter to transmit a desired wavelength band and exclude the photobleaching effect by all other wavelengths. OpenFS can be fabricated without any optical alignment because the 3D-printed housing autonomously aligns the optical components in OpenFS for precise positioning. A circuit diagram indicating the connections between the LED, spectrometer sensor, Arduino board, and Bluetooth module is displayed in Figure S1. A 1.5 mL microcentrifuge tube was used as a sample container; this provided the advantage of being inexpensive, disposable, and capable of measuring even with a small amount of sample (0.5 mL) compared to a cuvette (1.0 mL). In the assembled OpenFS, light from the LED reaches the center of the bottom of the tube and excites a fluorescence sample; then, the light emitted by the sample is transmitted to the sensor. The spectrometer sensor incorporates a linear CMOS image sensor (288 pixels) with a reflective grating that separates the incident light onto the pixels of the image sensor according to the wavelength. It outputs a sequence of spectral signals against the pixel number that must be converted into a wavelength using a calibration equation (eq 1) provided by the manufacturer. We measured the fluorescence intensities at a range of wavelengths (309.6−880.0 nm) by reading output signals from the spectrometer sensor using the Arduino board and transmitting the signals to a computer. Otherwise, the output signals were transmitted wirelessly to a smartphone (Galaxy S8+, Samsung Electronics Co., Ltd.) via the Bluetooth module and processed by a custom-developed Android application for graph display and data analysis. We call the former a "wired" type and the latter a "wireless" type. A portable power bank (10,400 mA h Li-ion battery) was used as an alternative power source to the computer for wireless operation. OpenFS can be modified to suit different applications by substituting elements such as a different wavelength light source or a narrower filter. The 3D printing design files for OpenFS and source codes for Arduino and Android are available on the journal website (see the Supporting Information) for open-source sharing of OpenFS and further modification by potential users. The STL file names for the 3D printing models are specified, and an assembly design is provided in Figure S2.

Software Application for Spectral Analysis

The spectrometer sensor measures the optical signal against the pixel number (n_p, 1−288) of the image sensor, which can be converted into the wavelength (λ) using the following equation:

$$\lambda = a_0 + b_1 n_p + b_2 {n_p}^2 + b_3 {n_p}^3 + b_4 {n_p}^4 + b_5 {n_p}^5 \quad (1)$$

where a_0, b_1, b_2, b_3, b_4, and b_5 are wavelength conversion factors whose values are 3.068464691×10^2, 2.707179585, $-1.45040638 \times 10^{-3}$, $-4.596809405 \times 10^{-6}$, $-3.105844784 \times 10^{-9}$, and $2.269126371 \times 10^{-11}$, respectively. The conversion factors vary with each sensor manufactured and are listed on a final inspection sheet provided by the manufacturer.

A customized Android application was developed for facile graph display and data analysis on a smartphone. The graphical user interface (GUI) of the application consists of graphical screens, spectral analysis, and data storage. After inserting a microcentrifuge tube containing a blank sample (distilled water in this paper), the "Blank" button is pressed to receive and display the blank signal. The "Sample" button is then pressed to obtain the fluorescence signal from a test sample (DNA sample in this paper), and the "Subtract" button is pressed to output the difference between the two signals as the final spectral result. By entering a desired wavelength at the bottom

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of the app, the user can check the fluorescence intensity at that wavelength. The result screen can be saved as an image by pressing the screenshot button, and the graph data can be saved as a text file by pressing the download button.

Sample Preparation

DNA oligonucleotides were purchased from Bioneer Corp. The sequences of the DNA oligos are listed in the Supporting Information (SI, see Table S3). DNA molecules were dissolved in distilled water at a concentration of 100 pmol/μL and stored in a refrigerator at −20 °C. For the experiments, thawed DNA samples were diluted to an appropriate concentration in a 1× Tris-EDTA buffer [50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, and 0.1 mM EDTA]. Hybridization was performed by mixing Cy3-labeled DNA probes, Cy5-labeled DNA probes, and target DNA molecules in a suitable ratio. The reactions were performed at 95 °C for 5 min, and the solution was then cooled slowly to room temperature.

Statistical Analysis

Data points and error bars in the figures represent the means and standard deviations from at least triplicate measurements. The limit of detection (LOD) is defined as $3\sigma/s$, and the limit of quantification (LOQ) is defined as $10\sigma/s$, where σ is the standard deviation of the blank, and s is the slope of the linear detection range. The dynamic range represents the concentration range between the LOQ and the limit of linearity (LOL). The R^2 value is obtained from linear regression. The sensitivity is defined as the slope of the calibration curve.

HAZARDS

Standard safety precautions in chemical laboratories should be followed, including suitable use of protective equipment such as a lab coat, gloves, and safety goggles. Since Cy3 and Cy5 dyes can cause skin and eye irritation, care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, a user should wash immediately with water. Used contents and containers must be disposed of in accordance with all local, regional, national, and international regulations.

RESULTS AND DISCUSSION

Device Principle

OpenFS was designed and fabricated by assembling all of the necessary electronics (i.e., Arduino microcontroller board and transistor) and optical components (i.e., LED and miniature spectral sensor) in a 3D-printed housing for fluorescence spectral analysis (Figure 1a). A light source, an LED, was arranged below at a 90° angle with the spectral sensor, and a sample tube could be placed at the intersection, preventing direct radiation from reaching the sensor (Figure 1b). We tested two different LED light sources (nominally called blue and cyan LEDs) to excite Cy3, not Cy5. Due to the wide excitation spectra of the dyes, an optimal excitation spectrum range for FRET can be between 440 and 500 nm, and only a part of the spectrum of each LED light source overlaps with the optimal excitation range (Figure S4).[29] Although both LEDs can be adjusted to similar intensity levels after bandpass filtering, the blue LED was used for the following experiments without the risk of Cy5 excitation by accidental light leakage. The blue LED, which is readily available, was used in this work, and their emission wavelength ranges did not perfectly match the excitation wavelength of Cy3. A light source with a peak emission wavelength of ∼475 nm would be optimal for

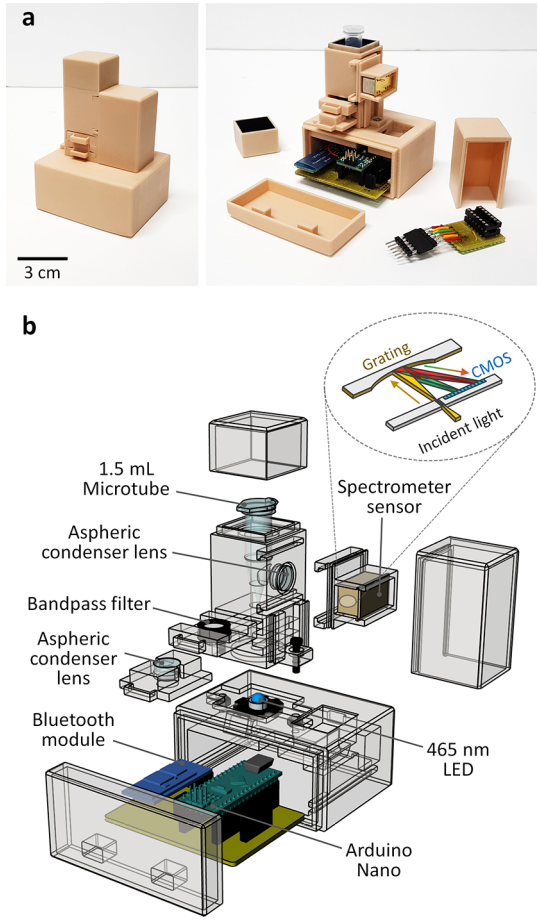

Figure 1. Open-source fluorescence emission spectrometer (OpenFS). (a) Photograph of OpenFS. The picture on the left displays the exterior of the finished product after assembly; the picture on the right displays the disassembled parts. (b) Schematic indicating the internal structure of OpenFS. The optical configuration indicating the distance between the internal components is shown in Figure S3.

OpenFS, but the combination of the blue LED and the condenser lens was able to provide sufficient excitation energy for Cy3 emission and FRET. The sensor was a fingertip-sized, high-sensitivity MEMS spectrometer sensor capable of detecting a range of wavelengths (309.6−880.0 nm). The signals from 500 to 800 nm, excluding the front light source wavelength band, were used for spectral analysis. OpenFS is based on an Arduino microcontroller that operates the LED and acquires fluorescence signals measured by the spectrometer sensor. OpenFS can be used even in a resource-limited setting without a computer by enabling wireless communication between the Arduino and a smartphone using a Bluetooth module. Simply connecting the USB cable from the Arduino board to a computer or a power bank enables fast and convenient fluorescence spectral analysis using an Arduino program or a custom Android application. The 3D printing design files and software source codes also facilitate open-source sharing of OpenFS and further modifications by potential users.

Spectral Analysis of Fluorescence Emission

Multispectral fluorescence analysis is essential for biological and biochemical experiments such as FRET; however, it relies on bulky, delicate, and expensive fluorescence spectrophotometers. OpenFS, a miniature, open-source, low-cost

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spectrophotometer can be useful for multispectral analysis applications, especially when laboratory access is limited or laboratory equipment is not available. We tested the analytical capability of OpenFS for fluorescence quantification. The experiment was conducted using a wired OpenFS with DNA molecules labeled with Cy3 fluorophore. The emission spectra were compared for different concentrations of the Cy3-labeled DNA probe (Figure 2a), and the spectral shape was in good agreement with the known Cy3 emission spectrum.[29] We compared the performance of OpenFS to measure the Cy3-labeled DNA probe with a conventional fluorometer (Denovix QFX Fluorometer, Denovix Inc.). Figure 2b,c indicates the fluorescence peak intensity of Cy3 versus the concentration of Cy3-labeled DNA measured using the wired OpenFS and the conventional fluorometer, respectively. OpenFS showed the LOD of 350 pM and dynamic range 1.16−160 nM, with R^2 = 0.9973 (n = 3). In comparison, the LOD of Denovix was calculated as 1.54 nM with R^2 = 0.9988 (n = 3), and the dynamic range was estimated to be from 5.12 nM to more than 640 nM. Although OpenFS showed a narrower dynamic range than the conventional fluorometer owing to the saturation of the spectrometer sensor, the dynamic range can be adjusted by changing the integration time of the sensor or by replacing the light source with LED sources of higher or lower light intensity.

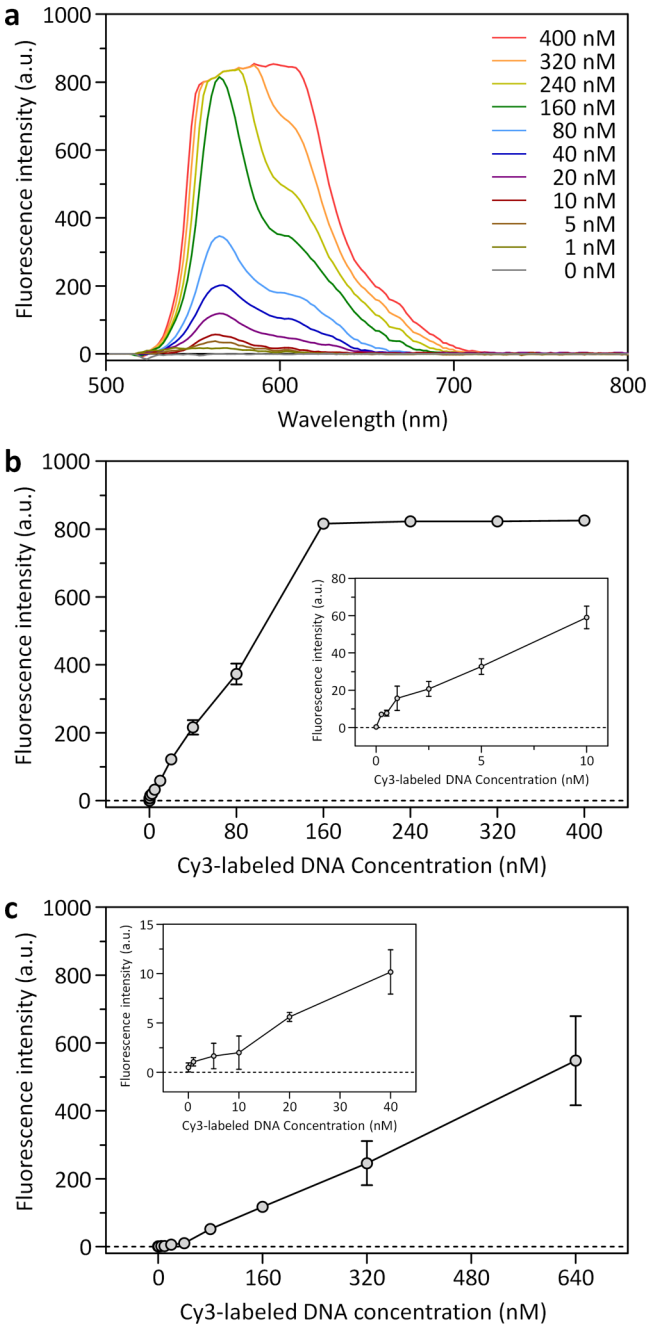

Figure 2. (a) Emission spectra of a Cy3-labeled DNA probe at a range of concentrations from 0 to 400 nM. (b) Fluorescence peak intensity of the DNA probe labeled with Cy3 at 565.2 nm according to the probe concentration (0−400 nM) measured using OpenFS. The inset displays the enlarged graph at low concentrations from 0 to 10 nM. (c) Fluorescence intensity according to the probe concentration (0−640 nM) measured using the conventional fluorometer. The inset displays the enlarged graph at low concentrations from 0 to 40 nM.

FRET-Based DNA Sensor

We demonstrated the utility of OpenFS to evaluate a FRET-based DNA sensor. The rapid and sensitive detection of nucleic acid targets is crucial in molecular diagnostics,[30] and FRET is an attractive tool as it enables the separation-free detection of low-abundance, unamplified DNA. As a model system, we adopted adjacent hybridization probes, which were oligonucleotides labeled with Cy3 fluorophore (550 nm excitation, 565 nm emission)[31] as a donor or Cy5 fluorophore (655 nm excitation, 667 nm emission)[31] as an acceptor. Cy3 and Cy5 are a well-known FRET pair in which the emission spectrum of Cy3 in the range between 530 and 690 nm and the absorption spectrum of Cy5 in the range between 510 and 690 nm overlap.[29] The DNA probes were designed so that Cy3 and Cy5 labels were placed close together on complementary target DNA (synthetic single-stranded oligonucleotide) after hybridization, causing fluorescence energy transfer from Cy3 to Cy5 (Figure 3a). We monitored the change in spectral signals with the concentration of the two probes fixed at 160 nM and the concentration of the target DNA changing from 0 to 160 nM. As indicated in Figure 3b, with the addition of the target DNA molecules, energy transfer between the Cy3 donor and Cy5 acceptor leads to a reduction in Cy3 emission and an increase in Cy5 emission, which is dependent on the target concentration. The degree of FRET was quantified by calculating the FRET efficiency (E_{FRET}), defined as follows:

$$E_{FRET} = I_A/(I_D + I_A) \quad (2)$$

where I_A and I_D are the fluorescence intensities of the acceptor and donor, respectively, measured using OpenFS at 668.9 and 565.2 nm, respectively. The E_{FRET} linearly increased up to the target DNA concentration of 160 nM, as Cy3−Cy5 pairs formed in proportion to the target molecules (Figure 3c). Therefore, the FRET efficiency (E_{FRET}) can be a measure to estimate the concentration of the target DNA molecules. From statistical analysis, the dynamic range was determined to be from 9.18 to 160 nM, with R^2 = 0.9905 (n = 3). The minimum target concentration at which fluorescence signals were distinguishable from a blank signal (or the LOD) was 2.75 nM. The performance of OpenFS with a FRET-based DNA sensor, such as the LOD, sensitivity, and dynamic range, can be further improved by changing the light source or adjusting the integration time of the spectrometer sensor.

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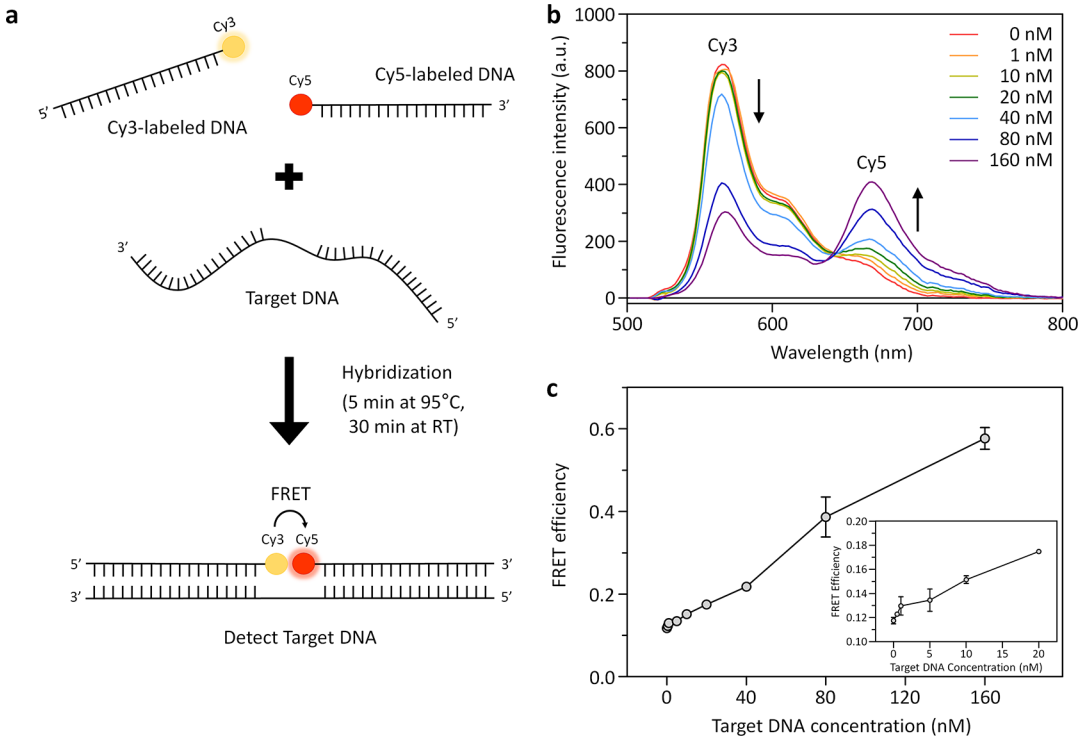

Figure 3. (a) Schematic showing the principle of a FRET-based DNA sensor. When a target DNA is hybridized with two different DNA probes labeled with Cy3 and Cy5, the distance between the fluorescent dyes becomes close, thereby causing fluorescence energy transfer from Cy3 to Cy5. (b) Changes in the emission spectra according to the target DNA concentration from 0 to 160 nM, at a fixed concentration (160 nM) of the DNA probes. Upon increasing the target DNA concentration, the fluorescence intensity at 565.2 nm (Cy3 peak) decreased, whereas the intensity at 668.9 nm (Cy5 peak) increased by FRET. (c) FRET efficiency (E_{FRET}) versus target DNA concentration (0−160 nM). The inset displays the enlarged graph at low concentrations of target DNA from 0 to 20 nM.

Integrating IoT Features into OpenFS

We transformed OpenFS into an IoT device by connecting it wirelessly to a smartphone and developing a dedicated Android application. A customized Android application was developed for quick graph display, convenient data analysis, and data storage on the smartphone (Figure 4a). By pressing the "Blank" and "Sample" buttons, the fluorescence signals of a blank sample (distilled water) and a test sample (DNA sample) were received, and the final emission spectrum, which is the difference between the sample and blank, could be obtained by pressing the "Subtract" button. After the measurement, a user could search for the fluorescence intensity at a desired wavelength, quickly check the automatically calculated FRET efficiency, and save the data for simple and accurate spectral analysis. Therefore, it is possible to conduct an OpenFS experiment wirelessly with minimal manipulation on the smartphone by simply connecting the Arduino cable to a power bank (Figure 4b). As tested with the FRET-based DNA sensor, there was no significant difference in detection performance between the wireless and wired OpenFS tested at the target concentration of 10 nM ($P = 0.0282$) and 80 nM ($P = 0.9860$), compared by a two-tailed unpaired t test ($P < 0.01$) (Figure 4c). Wireless OpenFS provides significant potential for converting fluorescence spectroscopy into a mobile healthcare platform by enabling wireless measurements and real-time analysis in a resource-limited setting.

Hands-On Exposure to OpenFS

We introduced OpenFS to undergraduate and graduate students through a training event in a classroom environment to assess the utility of OpenFS as an educational tool. OpenFS enables white-box testing so that students participating in the training event can gain an in-depth understanding of the relevant physical principles usually hidden in conventional black-box instruments. The training program included hands-on educational activities such as performing the FRET-based DNA assay using wireless OpenFS by following the directions on the student handout (see the Supporting Information). Eleven participants successfully detected target DNA using wireless OpenFS, by observing changes in the emission spectrum by FRET. After completing the experiments, a survey was conducted on the students (Table 1). The questionnaire was composed of seven items related to OpenFS: experience, usability, portability, speed (efficiency), accuracy, necessity, and recommendation. The Likert scale used in the survey ranges from 1 (strongly disagree) to 5 (strongly agree). As a result, 9 out of 11 students (81.8%) responded that they had no experience or were unfamiliar with fluorescence spectroscopy. Nevertheless, they responded that they fully understood and utilized OpenFS, giving high scores (average scores >4.7) to all items except experience. The high level of satisfaction for experimental education using OpenFS ensures that it can be used as an open-science tool for educational experiments as well as a research-grade instrument even in the developing world.

Novelty and Pedagogical Strengths of OpenFS

For the device novelty, we note that although various portable spectrometers have been proposed, OpenFS is the first truly portable, open-source fluorescence spectrometer whose performance is comparable to benchtop counterparts in terms of wavelength resolution and reliability. The battery-

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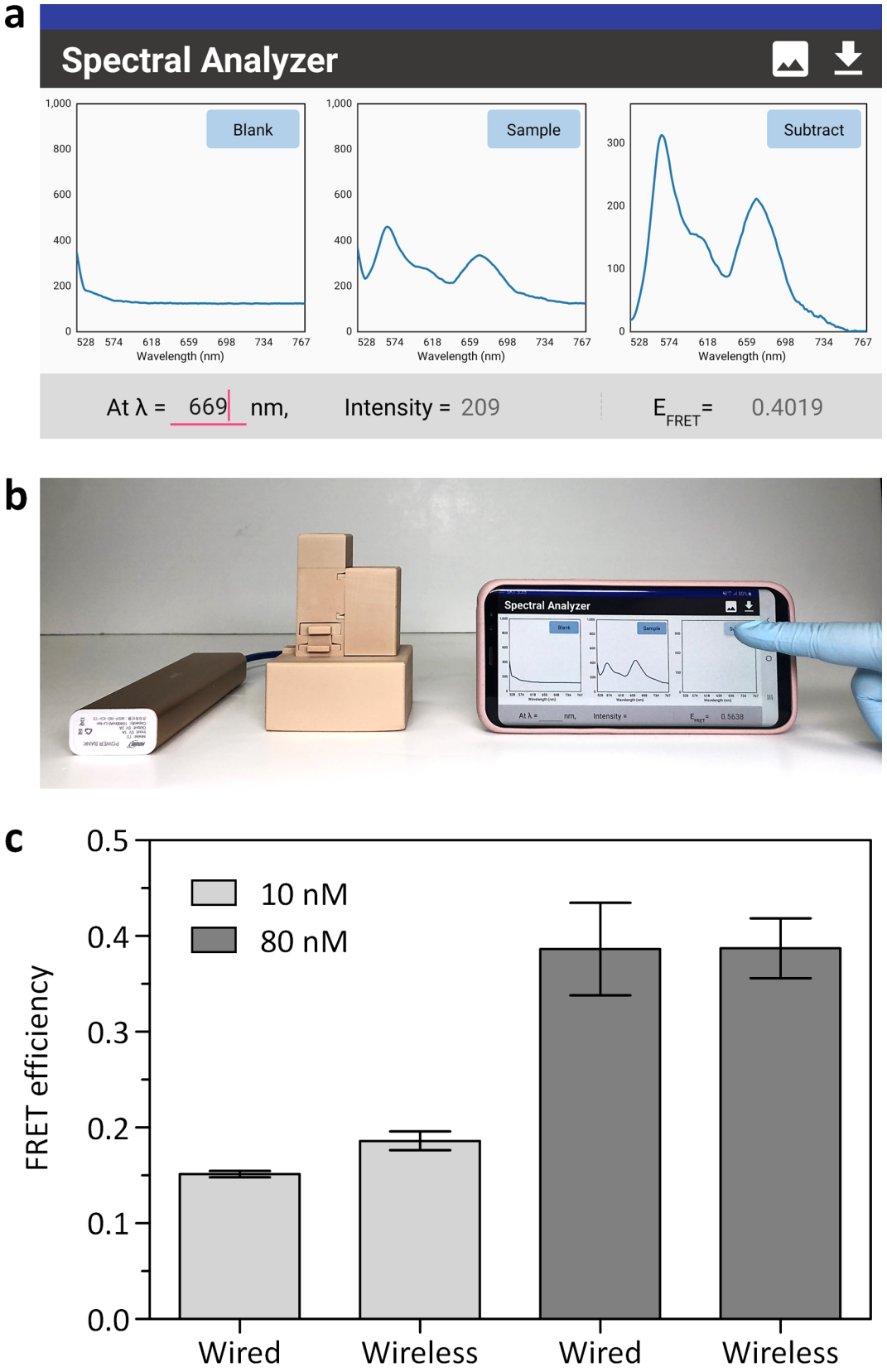

Figure 4. (a) Graphical user interface of the Android application that enables wireless, field-portable spectral analysis. (b) Wireless measurement using OpenFS, a power bank, and a smartphone. (c) Comparison of FRET efficiency (E_{FRET}) between the wired and wireless OpenFS, calculated at the target DNA concentrations of 10 and 80 nM.

powered spectrometer with smartphone readout enables portable and standalone operation, making it suitable for use in resource-limited applications and educational purposes. In addition to the device novelty, the pedagogical strengths of

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Table 1. Student Survey Results[a]

No.	Item	Average Score (N = 11)
1	Experience	1.91
2	Usability	4.73
3	Portability	5
4	Efficiency	4.82
5	Accuracy	4.82
6	Necessity	5
7	Recommendation	5

[a]The Likert scale for these scores has a range from 1 to 5, where 1 = strongly disagree, 2 = disagree, 3 = neutral, 4 = agree, and 5 = strongly agree. Detailed questions for each item are listed in the student handout (see the SI, Appendix).

OpenFS include (1) improved equipment accessibility by reduced fabrication cost and (2) easy fabrication and customization by a simple assembly of commercially available parts and 3D-printed housings. Therefore, OpenFS enables white-box testing so that students can build their own spectrometers and simultaneously gain an in-depth understanding of the relevant physical principles usually hidden in conventional black-box instruments. Because students can make OpenFS through various trial and error, these educational activities could increase their ability to fully inspect the open-source equipment and cultivate sustainable research and educational environments. The reliability in device design and performance ensures that OpenFS can be used as an open-science tool for educational experiments as well as a research-grade instrument in the developing world.

With the advantages of open-source technology and ease of fabrication, OpenFS can be customized simply by replacing LEDs and filters and changing 3D printing designs to extend the application of OpenFS. For example, bioparticles such as microorganisms can be detected through fluorescence detection or spectral change,[32,33] and the use of extrinsic fluorophores enables accurate quantification of DNA and RNA.[34] In industrial and environmental fields, OpenFS can be used to detect contaminating organic compounds and monitor wastewater.[35−38] For biomedical applications, OpenFS can be combined with nanoparticles and used for drug delivery, drug quality control, disease diagnosis, and biomedical imaging.[39−42]

CONCLUSION

We developed a compact open-source fluorescence spectrometer that implements a fast and efficient multispectral analysis. The spectrometer is assembled with commercially available electronic and optical elements in a 3D-printed housing, which allows easy modification of parts or designs to suit the user's purpose without any special technique or complex equipment. We demonstrated the capability of OpenFS as a sensitive fluorometer and a FRET-based DNA sensor. In addition, we demonstrated a simple and fast wireless spectral analysis using OpenFS by integrating a Bluetooth module and custom-developed Android application, thus proving the capability of OpenFS as an IoT device. The positive response of students who used OpenFS for experimental education proved its potential as an open-science tool for pedagogical instruction. Further replacement of optical components such as different wavelength light sources or filters enables fluorescence analysis from various types of samples in the biological, chemical, environmental, and pharmaceutical fields. We expect that OpenFS can be used as an open-source tool for incorporating fluorescence spectroscopy into numerous diverse applications beyond the fields wherein it is currently used.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.1c00560.

Circuit diagram, 3D printing models, optical configuration, and spectral measurement; cost analysis, comparison of fluorescence spectrometers, and DNA oligonucleotide sequences; student handout; supporting codes for Arduino source codes and Android Java code (PDF, DOCX)

Video of wireless spectral analysis (MOV)

3D printing design files (in STL format) used to construct OpenFS and the apk file of the Android application for wireless operation of OpenFS (ZIP)

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H.J. and S.S. contributed equally to this work.

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Selvin, P. R. The Renaissance of Fluorescence Resonance Energy Transfer. *Nat. Struct. Biol.* **2000**, 7 (9), 730−734.

(2) Lee, J.; Lee, S.; Ragunathan, K.; Joo, C.; Ha, T.; Hohng, S. Single-Molecule Four-Color FRET. *Angew. Chem., Int. Ed.* **2010**, *49* (51), 9922−9925.

(3) Sanchez, K. M.; Schlamadinger, D. E.; Gable, J. E.; Kim, J. E. Förster Resonance Energy Transfer and Conformational Stability of Proteins: An Advanced Biophysical Module for Physical Chemistry Students. *J. Chem. Educ.* **2008**, *85* (9), 1253−1256.
(4) Pazhani, Y.; Horn, A. E.; Grado, L.; Kugel, J. F. Evaluating the Relationship between FRET Changes and Distance Changes Using DNA Length and Restriction Enzyme Specificity. *J. Chem. Educ.* **2016**, *93* (2), 383−386.
(5) Nishimura, T.; Ogura, Y.; Yamada, K.; Ohno, Y.; Tanida, J. Biomolecule-to-Fluorescent-Color Encoder: Modulation of Fluorescence Emission via DNA Structural Changes. *Biomed. Opt. Express* **2014**, *5* (7), 2082.
(6) Massey, M.; Medintz, I. L.; Ancona, M. G.; Algar, W. R. Time-Gated FRET and DNA-Based Photonic Molecular Logic Gates: AND, OR, NAND, and NOR. *ACS Sensors* **2017**, *2* (8), 1205−1214.
(7) Zhang, C. Y.; Yeh, H. C.; Kuroki, M. T.; Wang, T. H. Single-Quantum-Dot-Based DNA Nanosensor. *Nat. Mater.* **2005**, *4* (11), 826−831.
(8) Goss, K. C.; Messier, G. G.; Potter, M. E. Data Detection Algorithms for Multiplexed Quantum Dot Encoding. *Opt. Express* **2012**, *20* (5), 5762.
(9) Geißler, D.; Charbonnière, L. J.; Ziessel, R. F.; Butlin, N. G.; Löhmannsröben, H. G.; Hildebrandt, N. Quantum Dot Biosensors for Ultrasensitive Multiplexed Diagnostic. *Angew. Chem., Int. Ed.* **2010**, *49* (8), 1396−1401.
(10) Millie, D.; Schofield, O.; Kirkpatrick, G.; Johnsen, G. Using Absorbance and Fluorescence Spectra to Discriminate Microalgae. *Eur. J. Phycol.* **2002**, *37*, 313.
(11) Wang, H.; Zhu, R.; Zhang, J.; Ni, L.; Shen, H.; Xie, P. A Novel and Convenient Method for Early Warning of Algal Cell Density by Chlorophyll Fluorescence Parameters and Its Application in a Highland Lake. *Front. Plant Sci.* **2018**, *9* (June), 1−13.
(12) Khan, S.; Zamyadi, A.; Rao, N. R. H.; Li, X.; Stuetz, R. M.; Henderson, R. K. Fluorescence Spectroscopic Characterisation of Algal Organic Matter: Towards Improved: In Situ Fluorometer Development. *Environ. Sci. Water Res. Technol.* **2019**, *5* (2), 417−432.
(13) Kim, H.; Kim, H. S.; Ha, J. S.; Lee, S. G. A Portable FRET Analyzer for Rapid Detection of Sugar Content. *Analyst* **2015**, *140* (10), 3384−3389.
(14) Porter, L. A.; Chapman, C. A.; Alaniz, J. A. Simple and Inexpensive 3D Printed Filter Fluorometer Designs: User-Friendly Instrument Models for Laboratory Learning and Outreach Activities. *J. Chem. Educ.* **2017**, *94* (1), 105−111.
(15) Kvittingen, E. V.; Kvittingen, L.; Melø, T. B.; Sjursnes, B. J.; Verley, R. Demonstrating Basic Properties of Spectroscopy Using a Self-Constructed Combined Fluorimeter and UV-Photometer. *J. Chem. Educ.* **2017**, *94* (10), 1486−1491.
(16) Yu, H.; Tan, Y.; Cunningham, B. T. Smartphone Fluorescence Spectroscopy. *Anal. Chem.* **2014**, *86* (17), 8805−8813.
(17) Arafat Hossain, M.; Canning, J.; Ast, S.; Cook, K.; Rutledge, P. J.; Jamalipour, A. Combined "Dual" Absorption and Fluorescence Smartphone Spectrometers. *Opt. Lett.* **2015**, *40* (8), 1737.
(18) Maia Chagas, A.; Prieto-Godino, L. L.; Arrenberg, A. B.; Baden, T. The €100 Lab: A 3D-Printable Open-Source Platform for Fluorescence Microscopy, Optogenetics, and Accurate Temperature Control during Behaviour of Zebrafish, Drosophila, and Caenorhabditis Elegans. *PLoS Biol.* **2017**, *15* (7), 437−440.
(19) Wigton, B. T.; Chohan, B. S.; McDonald, C.; Johnson, M.; Schunk, D.; Kreuter, R.; Sykes, D. A Portable, Low-Cost, LED Fluorimeter for Middle School, High School, and Undergraduate Chemistry Labs. *J. Chem. Educ.* **2011**, *88* (8), 1182−1187.
(20) Emmanuel, N.; Nair, R. B.; Abraham, B.; Yoosaf, K. Fabricating a Low-Cost Raman Spectrometer to Introduce Students to Spectroscopy Basics and Applied Instrument Design. *J. Chem. Educ.* **2021**, *98* (6), 2109−2116.
(21) Bhamla, M. S.; Benson, B.; Chai, C.; Katsikis, G.; Johri, A.; Prakash, M. Hand-Powered Ultralow-Cost Paper Centrifuge. *Nat. Biomed. Eng.* **2017**, *1* (1), 1−7.
(22) Lee, Y.; Kim, B.; Oh, I.; Choi, S. Optofluidic Modular Blocks for On-Demand and Open-Source Prototyping of Microfluidic Systems. *Small* **2018**, *14* (52), 1802769.
(23) Pereira, V. R.; Hosker, B. S. Low-Cost (<€5), Open-Source, Potential Alternative to Commercial Spectrophotometers. *PLoS Biol.* **2019**, *17* (6), e3000321.
(24) Kim, B.; Jeon, M. G.; Kim, Y. J.; Choi, S. Open-Source, Handheld, Wireless Spectrometer for Rapid Biochemical Assays. *Sens. Actuators, B* **2020**, *306*, 127537.
(25) Wittbrodt, B. T.; Squires, D. A.; Walbeck, J.; Campbell, E.; Campbell, W. H.; Pearce, J. M. Open-Source Photometric System for Enzymatic Nitrate Quantification. *PLoS One* **2015**, *10* (8), e0134989.
(26) Bogucki, R.; Greggila, M.; Mallory, P.; Feng, J.; Siman, K.; Khakipoor, B.; King, H.; Smith, A. W. A 3D-Printable Dual Beam Spectrophotometer with Multiplatform Smartphone Adaptor. *J. Chem. Educ.* **2019**, *96*, 1527.
(27) Kovarik, M. L.; Clapis, J. R.; Romano-Pringle, K. A. Review of Student-Built Spectroscopy Instrumentation Projects. *J. Chem. Educ.* **2020**, *97* (8), 2185−2195.
(28) Wigton, B. T.; Chohan, B. S.; Kreuter, R.; Sykes, D. The Characterization of an Easy-to-Operate Inexpensive Student-Built Fluorimeter. *J. Chem. Educ.* **2011**, *88* (8), 1188−1193.
(29) Tong, Q.; Zhu, Y.; Zhang, D.; Cai, Q.; Qu, W.; Cooper, C. D. O.; Wang, J.; McHugh, P. C. MicroRNA Quantitation during Dendritic Cell Endocytosis Using Imaging Flow Cytometry: Key Factors and Requirements. *Cell. Physiol. Biochem.* **2018**, *51* (2), 793−811.
(30) Sohrabi, N.; Valizadeh, A.; Farkhani, S. M.; Akbarzadeh, A. Basics of DNA Biosensors and Cancer Diagnosis. *Artif. Cells, Nanomed., Biotechnol.* **2016**, *44* (2), 654−663.
(31) Roy, R.; Hohng, S.; Ha, T. A Practical Guide to Single-Molecule FRET. *Nat. Methods* **2008**, *5* (6), 507−516.
(32) Shi, L.; Lu, L.; Harvey, G.; Harvey, T.; Rodríguez-Contreras, A.; Alfano, R. R. Label-Free Fluorescence Spectroscopy for Detecting Key Biomolecules in Brain Tissue from a Mouse Model of Alzheimer's Disease. *Sci. Rep.* **2017**, *7* (1), 1−7.
(33) Sohn, M.; Himmelsbach, D. S.; Barton, F. E.; Fedorka-Cray, P. J. Fluorescence Spectroscopy for Rapid Detection and Classification of Bacterial Pathogens. *Appl. Spectrosc.* **2009**, *63* (11), 1251−1255.
(34) Gallagher, S. R. Quantitation of DNA and RNA with Absorption and Fluorescence Spectroscopy. *Curr. Protoc. Immunol.* **2017**, *116*, A.3L.1−A.3L.14.
(35) Yu, H.; Song, Y.; Liu, R.; Pan, H.; Xiang, L.; Qian, F. Identifying Changes in Dissolved Organic Matter Content and Characteristics by Fluorescence Spectroscopy Coupled with Self-Organizing Map and Classification and Regression Tree Analysis during Wastewater Treatment. *Chemosphere* **2014**, *113*, 79−86.
(36) Mirnaghi, F. S.; Pinchin, N. P.; Yang, Z.; Hollebone, B. P.; Lambert, P.; Brown, C. E. Monitoring of Polycyclic Aromatic Hydrocarbon Contamination at Four Oil Spill Sites Using Fluorescence Spectroscopy Coupled with Parallel Factor-Principal Component Analysis. *Environ. Sci. Process. Impacts* **2019**, *21* (3), 413−426.
(37) Carstea, E. M.; Bridgeman, J.; Baker, A.; Reynolds, D. M. Fluorescence Spectroscopy for Wastewater Monitoring: A Review. *Water Res.* **2016**, *95*, 205−219.
(38) Wei, Q.; Yan, C.; Liu, J.; Luo, Z.; Xu, Q.; Zhang, X.; Chow, C. W. K.; Chong, M. N. Multistep, Microvolume Resin Fractionation Combined with 3D Fluorescence Spectroscopy for Improved DOM Characterization and Water Quality Monitoring. *Environ. Monit. Assess.* **2013**, *185* (4), 3233−3241.
(39) Nasimi, P.; Haidari, M. Medical Use of Nanoparticles: Drug Delivery and Diagnosis Diseases. *Int. J. Green Nanotechnol.* **2013**, *5* (1), 1−5.
(40) Wolfbeis, O. S. An Overview of Nanoparticles Commonly Used in Fluorescent Bioimaging. *Chem. Soc. Rev.* **2015**, *44* (14), 4743−4768.
(41) Lin, W. Introduction: Nanoparticles in Medicine. *Chem. Rev.* **2015**, *115* (19), 10407−10409.

https://doi.org/10.1021/acs.jchemed.1c00560
J. Chem. Educ. 2021, 98, 3493−3501

(42) Moreira, A. B.; Oliveira, H. P. M.; Atvars, T. D. Z.; Dias, I. L. T.; Neto, G. O.; Zagatto, E. A. G.; Kubota, L. T. Direct Determination of Paracetamol in Powdered Pharmaceutical Samples by Fluorescence Spectroscopy. *Anal. Chim. Acta* **2005**, *539* (1−2), 257−261.

https://doi.org/10.1021/acs.jchemed.1c00560
J. Chem. Educ. 2021, 98, 3493−3501