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"Open-Box" Approach to Measuring Fluorescence Quenching Using an iPad Screen and Digital SLR Camera

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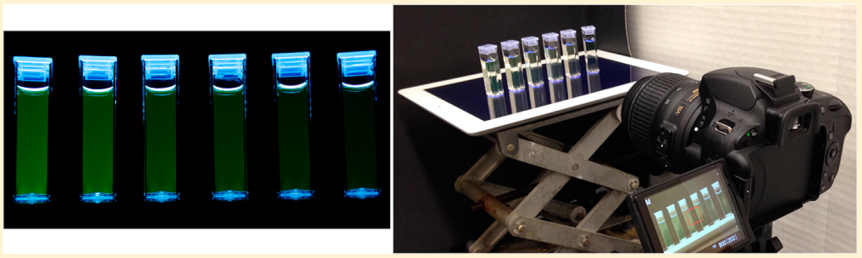
Supporting Information



ABSTRACT: Fluorescence quenching is an analytical technique and a common undergraduate laboratory exercise. Unfortunately, a typical quenching experiment requires the use of an expensive fluorometer that measures the relative fluorescence intensity of a single sample in a closed compartment unseen by the experimenter. To overcome these shortcomings, we designed an "open-box" fluorescence quenching method that uses an iPad screen as the excitation source and a digital single-lens reflex (SLR) camera as the detector. This setup enables a complete fluorescence quenching experiment to be performed and an accurate Stern−Volmer plot to be generated by analyzing a single image of six fluorescein samples and applying correction factors. The Stern−Volmer quenching constant (K_{SV}) calculated using this method was 9.62 ± 0.27 L mol^{-1}; fluorometer value, 9.52 ± 0.40 L mol^{-1}; literature values, 9.0 ± 0.2 L mol^{-1} and 9.608 L mol^{-1}. These results demonstrate that, in addition to allowing direct visualization of the chemical processes and simultaneous measurement of multiple samples, this simple method yields quantitative results comparable in accuracy to the more expensive fluorometer.

KEYWORDS: *Second-Year Undergraduate, Upper-Division Undergraduate, Fluorescence Spectroscopy, Physical Chemistry, Analogies/Transfer, Laboratory Equipment/Apparatus, Kinetics, Hands-On Learning/Manipulatives*

INTRODUCTION

Fluorescence is a common topic covered in undergraduate chemistry curricula. Unfortunately the cost of a typical research-grade fluorometer, in the range of tens of thousands of dollars, can be prohibitive. Another problem is that the advancements in fluorometers and the adaptation of highly sensitive instruments in teaching laboratories have buried all of the optics and electronics within a "black-box".[1−4] As a result, students' interactions with spectroscopic measurements have been reduced to pressing a button, and one of the advantages of fluorescence, the ability to visualize the emission of photons, has been lost. In addition, this inability to directly observe the sample during the data collection process understandably contributes to the difficulty in comprehending complex spectrophotometric concepts such as excitation spectra, emission spectra, and the Stokes shift.[5]

It is therefore not surprising that of the numerous new approaches to teaching fluorescence, many espouse developing demonstrations that highlight the visual aspects of this phenomenon.[1−7] One article describes a homemade "fluorescence microscope" made from poly(vinyl chloride) (PVC) pipe, a light-emitting diode (LED), and optical filters,[5] while another introduces a fluorometer made from a shoebox.[2] Although the educational importance of visualizing fluorescence cannot be overemphasized, a distinct deficiency of many of these experimental setups is that they typically yield *qualitative* rather than *quantitative* results.

Cost-effective and accurate instruments used to measure fluorescence have been described in the literature.[4,8] Similar to a fluorometer, however, these devices do not allow for maximum visualization of the samples during measurements. A technique that both yields accurate results and allows the student to fully visualize the excitation and emission of fluorescent compounds would be ideal.

We therefore designed an "open-box" method that enables students to both observe and accurately measure relative fluorescence intensity. Our method employs an iPad screen as the excitation source, a digital single-lens reflex (SLR) camera as the detector, and ImageJ or a MATLAB program to extract quantitative data from digital images. Utilizing varying concentrations of the quencher, iodide, we investigated the fluorescence quenching of fluorescein. While rivaling the accuracy of the more expensive fluorometer, and dramatically enhancing the students' educational experience, our method of measuring multiple samples simultaneously is also more efficient. In fact, our experimental design allows a complete

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fluorescence quenching experiment to be performed, and an accurate Stern−Volmer plot to be generated, by analyzing a single image and applying correction factors.

THEORY

Fluorescence

When a fluorescent molecule absorbs light, it transitions from its ground electronic state to an excited energy level. Once in an excited state, the molecule will ultimately relax back down to its ground state, while simultaneously emitting a photon. Typically, the excited singlet molecule will rapidly decay to the lowest vibrational level of the lowest singlet excited state, a process known as internal conversion.[9] As a result of this energy loss, the energy of the photon emitted will be less than the energy of the photon absorbed, a phenomenon known as the Stokes shift.

In our experiment, we investigated fluorescein, a common dye used for water tracing and coloring antifreeze. When excited by blue light, this molecule displays a green fluorescence. Since green light has a longer wavelength, and thus less energy, than blue light, the fluorescence of fluorescein is in keeping with the Stokes shift.

Quenching

Any process that decreases the fluorescence intensity and lifetime of a fluorophore is known as quenching.[10] In dynamic or bimolecular quenching, a second molecule collides with the fluorescent species and quenches or deactivates the excited state. Deactivation can occur by energy transfer, electron transfer, or catalytic deactivation of the excited state.

In our experiment, we used iodide to quench the fluorescence of fluorescein. The decrease in fluorescence intensity is described by the steady-state Stern−Volmer equation:[9]

$$F_0/F = 1 + K_{SV}[Q] \quad (1)$$

where F_0 is the fluorescence intensity in the absence of quencher, F is the measured fluorescence, and $[Q]$ denotes the concentration of the quencher in the solution. K_{SV} is called the Stern−Volmer quenching constant. If the ratio of the steady-state intensities against the quencher concentration is plotted, a line with a slope of K_{SV} is generated. K_{SV} corresponds to the sensitivity of a fluorescent molecule to a quencher and can be described by the following equation,[9] where k_Q is the bimolecular quenching rate constant and τ_0 is the unquenched lifetime:

$$K_{SV} = k_Q \tau_0 \quad (2)$$

OVERVIEW OF SETUP

A side-view of the experimental setup is shown in Figure 1. There were two main components: an iPad screen, used to excite the fluorescein samples, and a digital SLR camera, used as the detector of the fluorescence.

iPad Screen

A novel aspect of our experimental design was that we employed a blue iPad screen to excite fluorescein samples. We discovered that any blue liquid crystal display (LCD) screen was sufficient to excite fluorescein samples, and in earlier attempts we used a blue laptop screen as the excitation source. However, because this design resulted in inconsistent and irreproducible results (see Supporting Information for details on previous setups), we switched to an iPad screen. Because the iPad can be laid down flat, the fluorescein samples could be placed directly on the blue screen. This enabled excitation from under the cuvette, which allowed the detector to be perpendicular to the excitation source, thereby mimicking the setup of a fluorometer and minimizing spurious signals from scattered excitation. To enable the reproducible placement of the samples on the iPad screen without the need of an external stand, we used a paint application to create a screen that contained six blue squares each having the area of the bottom of a 1 cm cuvette (Figure 2). The remainder of the screen was black, which ensured that each sample received the same amount of blue light. Finally, we compensated for the change in viewing angle for the different cuvettes by rotating the outer blue squares so that the faces of the cuvettes appeared horizontally aligned in the images, as in Figure 4. Using a fluorometer, we determined that the blue squares emitted light at 447 nm, which provided sufficient excitation of the fluorescein samples (Figure 3).

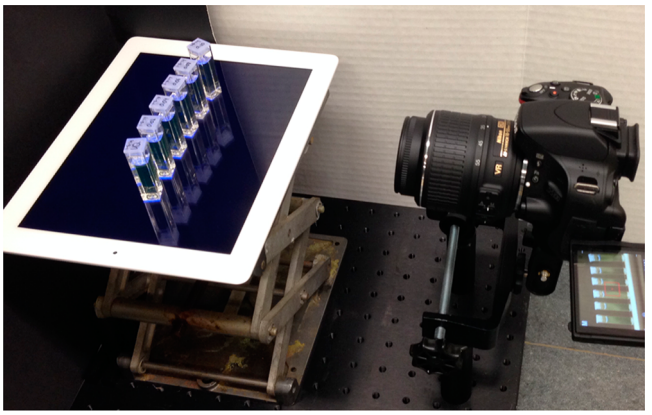

Figure 1. Side-view of experimental setup for our open-box approach to measuring fluorescence quenching. Components of the setup: six fluorescein samples with increasing iodide concentrations from left to right, iPad with custom-designed blue excitation screen (see Figure 2), and digital SLR camera (Nikon D5100). The center of the camera lens was slightly below the iPad screen and focused on the midpoint between the center two cuvettes, which were 16 cm away.

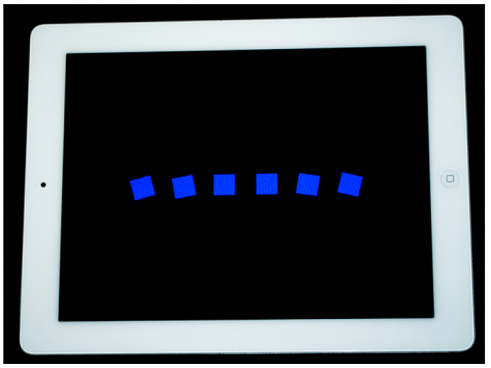

Figure 2. Custom-designed iPad excitation screen (excitation image is available for download in Supporting Information).

Digital Camera

We were able to accurately measure the fluorescence quenching of fluorescein because digital cameras are essentially three-color spectrographs and its sensor's output is linear. The camera

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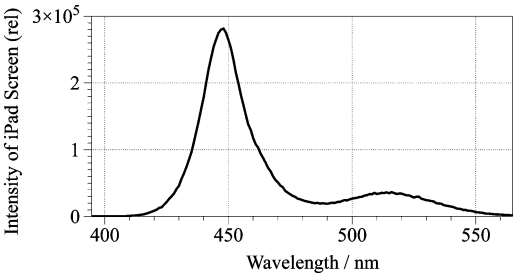

Figure 3. Emission spectrum of blue iPad screen measured using a Fluoromax-4 spectrofluorometer. Maximum intensity at 447 nm.

sensor is composed of an array of millions of photosites, commonly referred to as pixels. Pixels convert the incoming light into an electrical voltage that can be digitized by the sensor's analog to digital converter.[11] The voltage generated is directly proportional to the number of photons that hit a particular pixel on the sensor. For each pixel, the camera's internal computer assigns a brightness or intensity value that is dependent upon the bit-depth of the image. In order to produce a colored image, each pixel is covered with either a red, green, or blue filter and forms a pattern known as the Bayer filter mosaic.[11] Pixel intensities of the red, green, and blue channels are saved in each image and can be read by image analysis software. For instance, since fluorescein emits green light upon excitation, the green pixel intensity from an image of fluorescein will accurately reflect its relative fluorescence intensity.

■ EXPERIMENTAL METHOD

Materials

We used a Nikon D5100 DSLR camera with an 18−55 mm lens, an iPad 2, and 1 cm disposable acrylate cuvettes. The chemicals used were the free acid form of fluorescein (Sigma-Aldrich) and potassium iodide and sodium hydroxide (both AR grade).

Sample Preparation

A 1×10^{-6} M aqueous solution of unquenched fluorescein was prepared by dissolving its free acid form in a 0.1 M sodium hydroxide solution. To prepare a quenched fluorescein solution, potassium iodide was dissolved in the unquenched fluorescein solution to make a 0.10 M iodide solution. Various concentrations of quenched and unquenched fluorescein solutions were then mixed to produce the following concentrations of iodide: 0.02, 0.04, 0.06, and 0.08 M. These iodide solutions at 3.5 mL each, as well as the unquenched and 0.10 M iodide solution, were then transferred to six separate 1 cm disposable acrylate cuvettes. In addition, 3.5 mL aliquots of unquenched fluorescein solution were transferred to six different cuvettes to calculate the correction factors for the different positions on the iPad screen. Caps were placed securely on each cuvette in order to prevent loss of sample, reduce sample evaporation, and protect the iPad screen from sample spillage.

Setup

The iPad was placed on a flat surface in landscape mode. The screen was set to the maximum brightness level and the excitation image was opened. Six samples were placed on the iPad screen so that the bottom of the cuvettes completely and evenly covered each blue square. The digital SLR camera was placed on a stand with the center of the lens slightly lower than the iPad, so as to not directly detect any of the excitation light. The camera lens was located 16 cm from the two center cuvettes (cuvettes 3 and 4),and focused on the midpoint between these cuvettes. The camera was set to manual mode, ISO 400, f/5.6, a 2 s exposure time, and a 34 mm zoom. A remote control was used to initiate the capture of each image so as to avoid moving the camera. All images were taken in a dark room so that ambient light would not interfere with the results. For every image taken, both a JPEG and RAW image file were saved.

Capturing Images

Two different images were taken: the *quenching image* and the *correction image*. For the quenching image, the samples were placed on the blue squares of the iPad screen from left to right in order of increasing iodide concentration (Figure 4). In the correction image, six samples of unquenched fluorescein were placed on the blue squares. Care was taken to ensure that the camera, samples, and iPad screen were in identical positions for both the quenching and correction images.

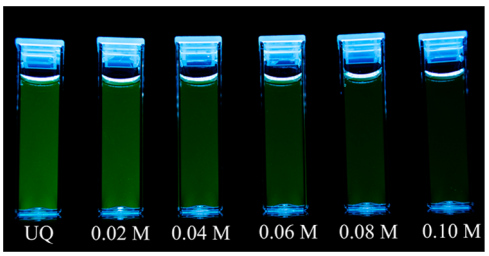

Figure 4. Quenching image: image of fluorescein samples with increasing iodide concentrations from left to right. UQ is the unquenched sample, and the values below each cuvette are the iodide concentrations. The decrease in emission intensity with increasing iodide concentration is clearly visible.

Image Conversion and Analysis

After the images were captured, the RAW image files were converted to linear 16-bit PPM files using the free command-line program, dcraw.[12,13] PPM files were then analyzed by one of two methods: ImageJ[14] or a MATLAB program. Both methods measured the average green pixel intensity of the bottom fourth of each cuvette. However, ImageJ is a manual process while the MATLAB program is automated.

Using the average green pixel intensities from the samples in the correction image, correction factors were calculated and applied to the average green pixel intensities of the samples in the quenching image, and F_0/F was plotted against the iodide concentration to generate a Stern−Volmer plot. A linear regression, with the y-intercept fixed at 1, was used to determine K_{SV}. The mean K_{SV} and standard deviation were calculated. Refer to the Supporting Information for further details regarding image conversion and analysis.

■ HAZARDS

Sodium hydroxide is a highly corrosive chemical. Contact with skin and eyes is hazardous. While fluorescein in its pure form has been reported to cause skin and eye irritation, few hazards exist for the low concentrations used in this experiment.

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RESULTS

Analysis of 88 16-bit PPM image files from eight sets of samples, and the subsequent application of correction factors, yielded a linear Stern–Volmer plot with a mean K_{SV} of 9.62 ± 0.27 L mol^{-1} (Figure 5). K_{SV} derived from our method correlated well with both our fluorometer (Fluoromax-4 spectrofluorometer) value of 9.52 ± 0.40 L mol^{-1} and the literature values of 9.0 ± 0.2 and 9.608 L mol^{-1}.[15,16]

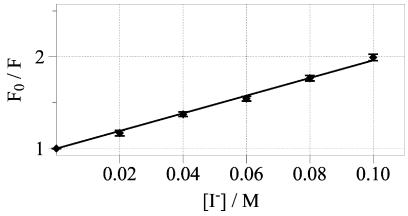

Figure 5. Stern–Volmer plot of the quenching of fluorescein by iodide using our open-box method. K_{SV} = 9.62 ± 0.27 L mol^{-1}. The plot was generated from the analysis of 88 images from eight sets of samples. The error bars on the plot represent ±1 standard deviation.

DISCUSSION

Comparison to a Fluorometer

Our approach to measuring fluorescence quenching has several advantages over a typical fluorometer. First, a fluorometer can cost tens of thousands of dollars, while the components of our setup cost approximately $1000. Second, our method measures the relative fluorescence intensities of six samples simultaneously, and the MATLAB program allows the automatic generation of a Stern–Volmer plot. In contrast, a fluorometer requires the measurement of the six samples individually, the manual exportation of the intensity data to a spreadsheet, followed by the construction of the Stern–Volmer plot. Finally, the results from our method are accurate and comparable to those of a fluorometer (K_{SV} = 9.62 ± 0.27 and 9.52 ± 0.40 L mol^{-1}, respectively). Thus, our method provides a more affordable, simpler, and efficient means to complete a fluorescence quenching experiment with comparable accuracy.

Our open-box method also has many educational advantages over a fluorometer. A fluorometer is a black-box instrument that hides the sample and inner parts inside an opaque compartment. Students have no view of the sample during the measurement and only indirectly manipulate the instrument with a computer. When the underlying chemistry is hidden from students, they begin to treat the experiment as a "data collection" exercise and not as a time to learn, understand, and put chemistry concepts into practice. Using our method, students have a complete and direct view of the samples throughout the entire experiment. They can see the blue excitation light from the iPad screen as well as the green emission from the fluorescein samples. No longer are the colors blue and green simply wavelengths that students input into a computer program. In addition, the samples' proximity to each other on the iPad screen allows students to compare the fluorescence of the samples and to visualize the correlation between quencher concentration and fluorescence intensity. Finally, the act of capturing the images and analyzing each cuvette with ImageJ allows students to perform the work of a fluorometer, thereby enhancing the understanding of the critical concepts of fluorescence and quenching.

Importance of Correction Factors

At the beginning of this project we attempted to attain accurate results solely by analyzing the quenching images. However, we soon realized that when a Stern–Volmer plot was generated by this method, the result was a curved rather than a linear plot (Figure 6). We hypothesized that this difference was secondary to the decreased sensitivity of the camera for detecting light from peripheral objects, a phenomenon known as vignetting. In this experimental setup, vignetting caused the pixel intensities of the samples to the left and right of the camera's center focal point to be lower than they should be, the impact of which increased as the distance from the focal point increased (Figure 7a). The end result was the curved plot seen in Figure 6.

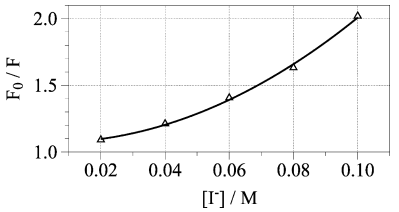

Figure 6. Effect of vignetting on the linearity of the Stern–Volmer plot. Data acquired by measuring the average green pixel intensities of the samples in the quenching image without application of correction factors.

To account for this inaccuracy, we introduced the correction image, an image of six unquenched fluorescein samples arranged on the iPad screen identically to the quenched samples. The principle behind the correction image is that the pixel intensities of the cuvettes in an image of six identical fluorescein samples should be the same, and any difference is attributed to vignetting. Figure 7a shows the average green pixel intensity measured from each cuvette in the correction image. The intensity differences between the samples are substantial and will vary with lens, camera, and focal distance. To account for vignetting, we utilized the correction image to calculate correction factors for each of the six positions on the iPad screen, giving the cuvette with maximum intensity a value of 1 (Figure 7b). The average green pixel intensity of the corresponding cuvette in the quenching image was then divided by this correction factor. Since the degree of vignetting depends upon the position of the camera in relation to the samples, the accuracy of this correction required that the setup be identical for both the correction and quenching images. When comparing Figures 5 and 6, it is clear that the application of correction factors improved the linearity of the Stern–Volmer plot and was critical to obtaining accurate results.

Theoretical Importance of Analyzing Linear Images

While camera sensors detect light linearly, human perception of brightness is logarithmic. To compensate for this difference, so images appear "natural" to the human eye, cameras automatically apply gamma correction when processing data from the sensor. Gamma correction, or simply gamma, is a nonlinear operation defined by the following equation:[11] $V_{out} = V_{in}^{gamma}$, where V_{out} is the output luminance value and V_{in} is the input or actual luminance value, with luminance being the amount of light emitted from an object or area. The standard value for gamma is 1/2.2.[11] When gamma correction is applied, pixel intensities are altered and no longer directly relate to the number of photons that hit the camera sensor. The image is

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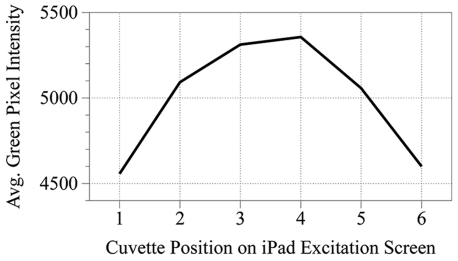

	A	B	C
1	POSITION	INTENSITY	Corr. Factor
2	1	4556.323	0.85070146
3	2	5092.063	0.95072835
4	3	5312.292	0.99184684
5	4	5355.96	1
6	5	5057.748	0.94432147
7	6	4600.372	0.85892576

Figure 7. (a, left) Effect of vignetting on average green pixel intensity of six identical fluorescein samples and (b, right) derivation of correction factors (data from single trial). Position 1 is farthest to the left; position 6 is farthest to the right.

considered "nonlinear", and any quantitative information gathered from it would be inaccurate.

To avoid the inaccuracies that accompany nonlinear operations such as gamma correction, one must be cognizant of exactly what changes are applied to an image from the time it is captured to when it is analyzed. Many cameras have the capability of saving RAW image files, which contain unprocessed and unaltered pixel information from the camera sensor. To acquire accurate quantitative data from such images, however, one must convert these RAW files to a readable format using a *linear* RAW file converter, such as dcraw. This approach maintains the linearity and consequently the scientific accuracy of the image. If instead, one uses an alternative method to convert a RAW file to another format, such as JPEG or TIFF, gamma correction or other operations could be applied, sometimes unknowingly. A process as simple as reading a RAW image into Photoshop immediately, and without warning, applies a distorting correction. The result would be a nonlinear image, unsuitable for scientific investigations.

Impact of Nonlinear vs Linear Images on Results

Since our goal was to create a system with accuracy similar to that of a fluorometer, it was critical to ensure that our method's conversion program, dcraw, preserved the linearity from the camera sensor when it converted the RAW images to an analyzable format. To test the linearity of images converted using dcraw and compare it to images from other conversion processes, we took images of a uniformly lighted white piece of paper with a constant aperture and ISO at various exposure times ranging from 1/10 to 10 s. Exposure time is the duration of time in which the camera sensor is exposed to light. Given the camera sensor's linear nature, a plot of pixel intensity versus exposure time should be a straight line. The RAW image files of the paper were converted to four different file formats using three separate conversion methods: (1) 8-bit JPEG (Joint Photographic Experts Group) files using an internal camera conversion, (2) 16-bit TIFF (Tagged Image File Format) files using the Nikon software, ViewNX 2, and (3) 8-bit and 16-bit PPM (Portable Pixel Map) files using dcraw. We subsequently measured the average green pixel intensity of each image and plotted those values against the corresponding exposure time (Figure 8).

As parts b and d of Figure 8 illustrate, pixel intensity is directly proportional to the exposure time, resulting in linear plots. This result confirms that converting the RAW image files to 8-bit and 16-bit PPM files using dcraw does not alter pixel information from the camera sensor and is therefore a suitable conversion process for our method. In contrast, when the JPEG and TIFF files were analyzed (Figure 8a,c), the pixel intensities did not increase linearly with exposure time. The reason for this

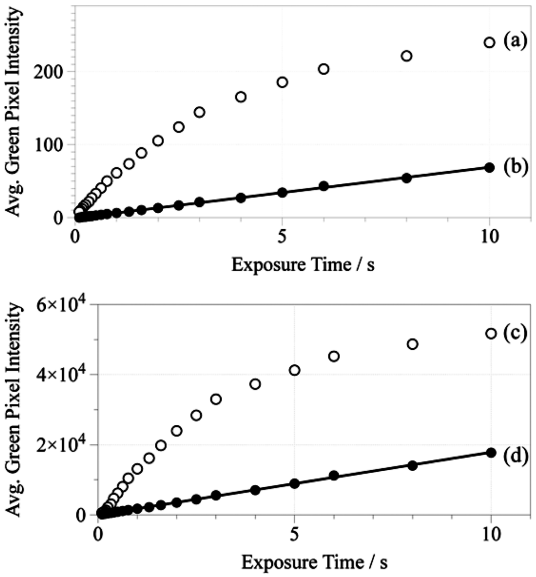

Figure 8. Relationship between exposure time and average green pixel intensity for JPEG [internal camera conversion] (a), 8-bit PPM [dcraw conversion] (b), 16-bit TIFF [ViewNX 2 conversion] (c), and 16-bit PPM [dcraw conversion] (d) files. The solid lines in both plots represent the linear fits of b and d, respectively.

lack of linearity of JPEG and TIFF files is that the conversion processes used for these images applied gamma correction, resulting in pixel intensities no longer accurately reflecting the amount of light the camera sensor detected.

To demonstrate the impact analyzing nonlinear images can have on K_{SV} values determined by our method, we took quenching and correction images at exposure times ranging from 1/10 to 10 s at a constant aperture and ISO. RAW image files were then converted to nonlinear JPEG and TIFF files and linear PPM files. Correction factors were calculated and applied to the average green pixel intensities of the samples in the quenching images, Stern−Volmer plots were generated, and K_{SV} values were determined. Plotting K_{SV} versus exposure time (Figure 9) clearly revealed that when nonlinear JPEG and TIFF files were utilized, K_{SV} was highly dependent on exposure time, decreasing almost an order of magnitude as exposure time increased (Figure 9b). In contrast, with dcraw conversion, K_{SV} was independent of the exposure time (Figure 9a), confirming the necessity of analyzing linear images to obtain accurate and reproducible fluorescence quenching results.

Numerous papers that describe using digital images to acquire scientific data claim that any file format, including JPEG, can be analyzed and still yield accurate results.[17−20] However, as illustrated by Figures 8 and 9, such assertions are

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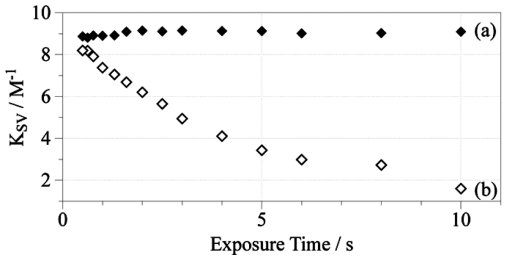

Figure 9. Effect of linear (a) and nonlinear (b) image analysis on K_{SV}.

incorrect because any process that applies nonlinear operations, such as gamma correction (such as occurs with conversion to a JPEG file format), adversely impacts the experimental outcome. One example is the investigation published by Cumberbatch and Hanley. These authors described a quantitative method for iodide quenching of fluorescein using a digital camera as the detector and a UV transilluminator as the excitation source.[17] In this work, K_{SV} varied from 5.4 to 11.8 L mol^{-1}.[17] As Figure 9b demonstrates, one possible explanation for this wide range of K_{SV} values is that the authors did not take into account the impact analyzing nonlinear images, such as JPEG files, could have on the precision and accuracy of their results.

CONCLUSION

We described a fluorescence quenching method using a digital SLR camera and an iPad screen that is comparable in accuracy to a fluorometer, yet has the added benefits of affordability, efficiency, and visualization. The calculation and implementation of correction factors allows one to accurately perform a fluorescence quenching experiment by taking one image of six samples simultaneously. Because of the open-box design of our setup, students can visualize the excitation and emission of the fluorescein samples, which will lead to a better understanding of fluorescence and quenching. By using ImageJ, students can become completely involved in the data analysis. Alternatively, the automated MATLAB program enables the rapid analysis of many images and the automatic generation of a Stern−Volmer plot for each.

We demonstrated that in order to accurately use digital images for quantitative measurements, a suitable RAW file converter must be employed to preserve the linearity of an image. JPEG images and other nonlinear image types should be avoided.

Finally, this experimental method is not limited to measuring only the fluorescence quenching of fluorescein, but has numerous other applications. For instance, employing another MATLAB program we designed, one can determine an unknown quencher concentration (see Supporting Information for details). By utilizing the polarization of LCD screens and placing a polarizer over the camera lens, one can potentially make anisotropy measurements. Additionally, one can measure the fluorescence quenching of other fluorophores by simply changing the color of the excitation squares on the iPad screen and analyzing the pixel channel related to the color of the emission. Rhodamines, for example, can be excited with visible radiation and viewed on a digital camera.

One limitation of using a digital camera as the detector, however, is that it can only measure molecules that fluoresce red, green, or blue light. As a result of this constraint, we do not claim that this open-box method eradicates the need for fluorometers. Rather, we assert only that this method provides a unique alternative to teaching and measuring fluorescence quenching, one that will be particularly beneficial to those laboratories that do not have access to a fluorometer.

ASSOCIATED CONTENT

Supporting Information

Details regarding previous setups, notes, and potentially helpful tips, image conversion and analysis steps, MATLAB programs, and more, additional files containing the excitation screen image, code for the MATLAB programs, sample RAW correction and quenching images, and a sample Excel document. This material is available via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

REFERENCES

(1) Delorenzi, N. J.; Araujo, C.; Palozzolo, G.; Gatti, C. A. A Simple Device to Demonstrate the Principles of Fluorometry. *J. Chem. Educ.* **1999**, *76*, 1265−1266.
(2) Wahab, M. F. Fluorescence Spectroscopy in a Shoebox. *J. Chem. Educ.* **2007**, *84*, 1308−1312.
(3) Scheeline, A.; Kelley, K. Cell Phone Spectrometer: Learning Spectrophotometry by Building and Characterizing an Instrument. (Published Nov. 2009). http://www.asdlib.org/onlineArticles/elabware/Scheeline_Kelly_Spectrophotometer/Cell%20Phone%20Spectrometer%20Paper.pdf (accessed Nov. 2013).
(4) Wilczek-Vera, G.; Salin, E. D. Understanding Fluorescence Measurements through a Guided-Inquiry and Discovery Experiment in Advanced Analytical Laboratory. *J. Chem. Educ.* **2011**, *88*, 216−219.
(5) LaFratta, C. N.; Huh, S. H.; Mallillin, A. C.; Riviello, P. J.; Walt, D. R. Visualizing Fluorescence: Using a Homemade Fluorescence "Microscope" To View Latent Figerprints on Paper. *J. Chem. Educ.* **2010**, *87*, 1105−1107.
(6) Croney, J. C.; Jameson, D. M.; Learmonth, R. P. Fluorescence spectroscopy in biochemistry: Teaching basic principles with visual demonstrations. *Biochem. Mol. Biol. Educ.* **2001**, *29*, 60−65.
(7) MacCormac, A.; O'Brien, E.; O'Kennedy, R. Classroom Activity Connections: Lessons from Fluorescence. *J. Chem. Educ.* **2010**, *87*, 685−687.
(8) Bare, W. D.; Pham, C. V.; Cuber, M.; Demas, J. N. An Improved Method for Studying the Enzyme-Catalyzed Oxidation of Glucose using Luminescent Probes. *J. Chem. Educ.* **2007**, *84*, 1511−1514.
(9) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*, 3rd ed.; Springer Science + Business Media: New York, 2006.
(10) Fraiji, L. K.; Hayes, D. M.; Werner, T. C. Static and Dynamic Fluorescence Quenching Experiments for the Physical Chemistry Laboratory. *J. Chem. Educ.* **1992**, *69*, 424−428.
(11) Cambridge in Colour. http://www.cambridgeincolour.com/ (accessed November 2013).
(12) Coffin, D. Decoding raw digital photos in Linux. http://www.cybercom.net/~dcoffin/dcraw/ (Accessed November 2013).
(13) Johnson, M. K. Capturing Linear Images. http://www.mit.edu/~kimo/blog/linear.html (accessed April 2013).
(14) ImageJ. http://rsb.info.nih.gov/ij/ (accessed November 2012).
(15) Watkins, A. N.; Ingersoll, C. M.; Baker, G. A.; Bright, F. V. A Parallel Multiharmonic Frequency-Domain Fluorometer for Measuring Excited-State Decay Kinetics Following One-, Two-, or Three-Photon Excitation. *Anal. Chem.* **1998**, *70*, 3384−3396.
(16) Albani, J. R. Dynamics of the Lens culinaris agglutinin-lactotransferrin and serotransferrin complexes, followed by fluores-

cence intensity quenching of fluorescein (FITC) with iodide and temperature. *Biochim. Biophys. Acta* **1998**, *1425*, 405−410.
(17) Cumberbatch, T.; Hanley, Q. S. Quantitative Imaging in the Laboratory: Fast Kinetics and Fluorescence Quenching. *J. Chem. Educ.* **2007**, *84*, 1319−1322.
(18) Kohl, S. K.; Landmark, J. D.; Stickle, D. F. Demonstration of Absorbance Using Digital Color Image Analysis and Colored Solutions. *J. Chem. Educ.* **2006**, *83*, 644−646.
(19) Kehoe, E.; Penn, R. L. Introducing Colorimetric Analysis with Camera Phones and Digital Cameras: An Activity for High School or General Chemistry. *J. Chem. Educ.* **2013**, *90*, 1191−1195.
(20) Quagliano, J. M.; Marks, C. A. Demystifying Spectroscopy with Secondary Students: Designing and Using a Custom-Built Spectrometer. *J. Chem. Educ.* **2013**, *90*, 1409−1410.