Inexpensive, Open Source Filter Fluorometers for Measuring Relative Fluorescence

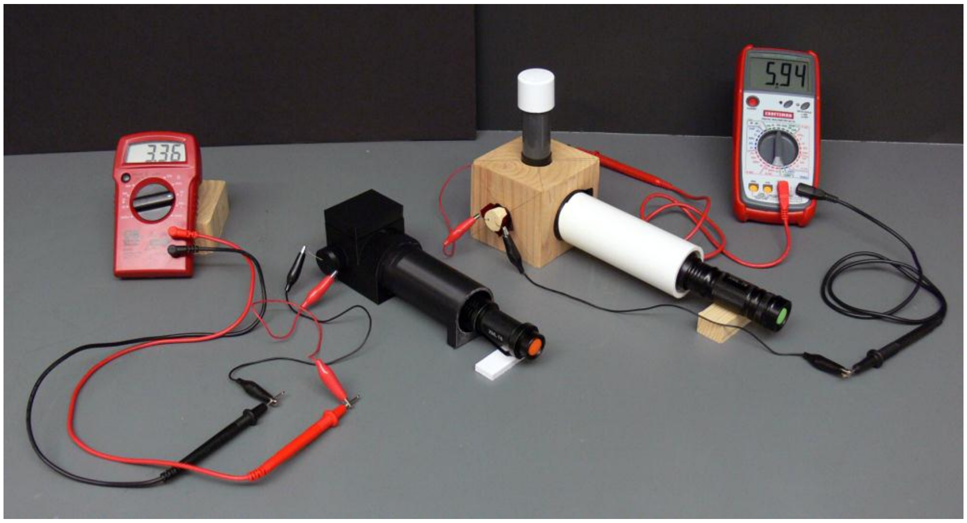
Chris Stewart and John Giannini *

Biology Department, St. Olaf College, 1520 St. Olaf Avenue, Northfield, MN 55057

ABSTRACT

Expanding upon the work of other teams, we provide two different designs for making inexpensive, single beam, filter fluorometers that can be used to measure the relative fluorescence of different chemicals in solution and demonstrate basic principles of fluorometry. Like other instruments that we have developed, the first model can be assembled from 3D-printed parts, and the second version can be built using supplies available at most hardware stores or online. Both models use: (i) a sensitive light dependent resistor (LDR) connected to a digital multimeter as their detector; (ii) a tactical LED flashlight with a convex lens and adjustable head to focus the beam as the light source; and (iii) pieces of colored cellophane as their excitation and emission filters. In addition, both fluorometers contain a 4x objective lens from a compound microscope to further focus the light beam and increase its intensity. We tested these models using increasing concentrations of two common fluorophores (Rhodamine B and Acridine Orange) in solution, and we found that the instruments generated data and trends similar to those of other devices described in the literature. We further explain how these fluorometers can be used in a chemistry, biochemistry, biology, or physics course to illustrate some of the basic principles of fluorometry, such as how these instruments are designed and built and how the intensity of a fluorophore in solution varies with its concentration. As with other low-cost and open source designs that we have developed, we have named these instruments “OPN Fluorometers”, and we explain how to 3D print or build these devices in the Supporting Information in hopes that others will be able to use or modify these plans to fit their educational or other needs.

ABSTRACT GRAPHIC



KEYWORDS

General Public, Analytical Chemistry, Biochemistry, Laboratory Instruction, Hands-On Learning / Manipulatives, Laboratory Equipment / Apparatus, Photochemistry, Fluorescence Spectroscopy, Fluorometry

INTRODUCTION

In a series of recent papers, we described how to 3D print or build inexpensive epifluorescence microscopes, colorimeters, and spectrophotometers for educational or other purposes.[1-3] We named these instruments “OPN Scopes,” “OPN Colorimeters,” and “OPN Specs” because their plans and parts were designed to be open and accessible to all. Using this approach, we have developed other low-cost scientific equipment as well (e.g., micro-pipettes, mini-centrifuges, clinical centrifuges, and low-temperature incubators),[4] and we describe here two ways to make inexpensive, single beam filter fluorometers for use in the classroom or teaching lab to explore relative fluorescence.

In general, a filter fluorometer is a device that measures the concentration of fluorescent chemicals (called “fluorophores”) in solution. In a sense, the instrument is a cross between an epifluorescence microscope and a colorimeter or spectrophotometer.

Like an epifluorescence microscope,[1] a filter fluorometer works by sending light through an optical filter (called an "excitation filter") in order to transmit a specific wavelength into a sample, which then excites electrons in the specimen – i.e., raises them to a higher energy level.[5-8] As those electrons fall back to their initial (i.e., ground) state, they release light energy at a slightly longer wavelength (and, thus, one at a lower energy and with a different color), which is called fluorescence.[5-8] That fluoresced (or emitted) light then passes through another optical filter (called an "emission filter") before it reaches a detector that measures the intensity of the emitted (i.e., fluorescent) light.

However, unlike an epifluorescence microscope, which transmits its light from above,[1] a fluorometer sends in its beam of light into the sample from the front (similar to the way in which a colorimeter or spectrophotometer works).[5-8] But, instead of placing the sensor directly in the path of the light beam, as would occur in a colorimeter or spectrophotometer,[2, 3] a fluorometer actually measures the intensity of the fluorescent light at a 90° angle (Fig. 1), which helps to reduce any interference from the light beam (or other background radiation) and decrease the amount of light scattering due to the solution and any substances contained within it.[5-8] Since fluorescent light radiates out in all directions from the sample,[5-8] taking these measurements at a 90° angle is not a limiting factor of the instrument. In fact, this 90° angle is often the optimal one for recording the intensity of emitted fluorescent light.[5-8]

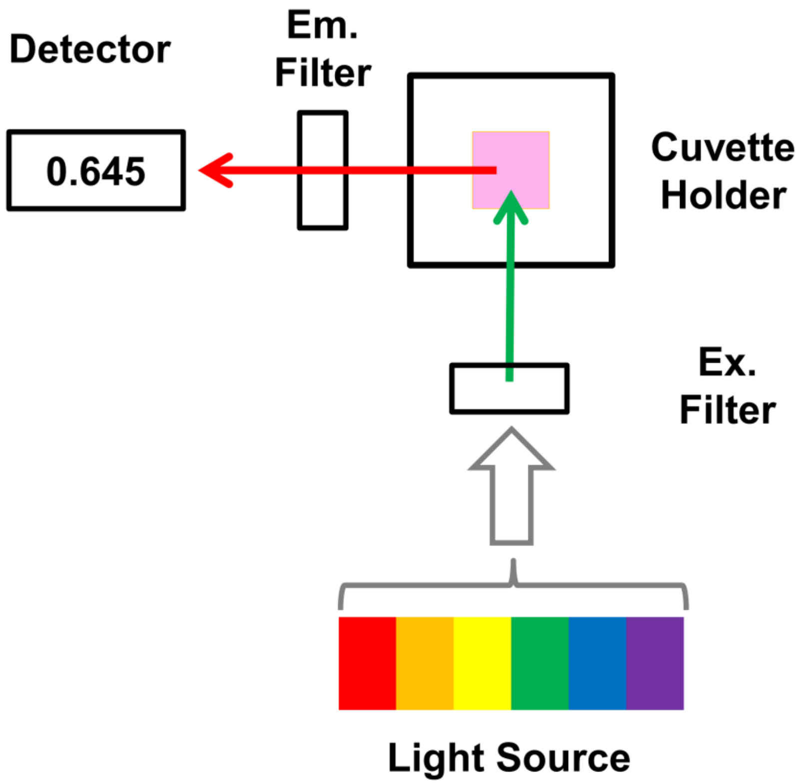


Figure 1. The workings of a basic filter fluorometer. A high-powered light source transmits an intense white light through an excitation filter, which screens out all but a narrow band of wavelengths to excite electrons in the sample (bottom). When those electrons fall back down to their ground state, they emit light at a longer (and lower energy) wavelength, which is known as "fluorescence" (top center). That emitted light then passes through an emission filter, which allows only the emitted wavelength(s) to pass, before they ultimately reach a detector, which measures the intensity of that light (top left).

To illustrate these concepts, consider the following set-up designed to measure the fluorescence of a solution containing the common fluorophore Rhodamine B (Fig. 1). First, a clear cuvette is placed in the fluorometer (Fig. 1, top). A clear cuvette is used because it will later allow any emitted fluorescent light to radiate out in all directions.[5-8] Then, a sample of Rhodamine B solution is placed into the cuvette, and an intense white light is subsequently sent through an excitation filter, which allows only a narrow band in the green spectrum to pass (Fig. 1, bottom).[5-8] That green light then strikes the sample solution, exciting electrons in the Rhodamine B molecules (Fig. 1, top). As those electrons fall back to their ground state, they release light energy at a longer (and lower energy) wavelength,[5-8] which radiates out in all directions and is typically seen as a faint red or orange glow (Fig. 1, top). That light then passes through an emission filter, which is placed at a 90° angle to the cuvette in order to reduce the amount of interference from the light beam and other sources (Fig. 1, left).[5-8] In particular, this emission filter allows only the emitted fluorescent light to pass, and this light ultimately reaches a detector, which measures the intensity of that emitted light (Fig. 1, far left).[5-8]

Of course, not all fluorometers are filter fluorometers, which use optical filters to screen their excitation and emission wavelengths (like the model described above).[5-8] Instead, like spectrophotometers,[3] other fluorometers use monochromators to generate their excitation wavelength(s) and to filter the emitted fluorescent light before it reaches the detector.[5-8] Not surprisingly, these instruments are called "spectrofluorometers" because, like a spectrophotometer, they are capable of generating an entire spectrum. Then, they select one or more specific wavelengths to send into a sample while using another monochromator to filter the emitted light before it reaches a detector.

Despite their importance as analytical tools, commercial fluorometers and spectrofluorometers are often too expensive to use in a classroom or teaching lab,[9-12] especially given the limited budgets of many educational institutions. In addition, as with other scientific instruments,[1-3] due to the way in which many fluorometers are designed and used, students frequently see these devices as "black boxes," which offer little insight into how they actually work.[9-11, 13-15] In an effort to address these issues, various teams have proposed some very insightful and innovative designs for educational fluorometers over the years.[9-24] These models range from the relatively straightforward (e.g., those made from a cardboard shoebox and which use pieces of colored cellophane for its filters or part of a CD or DVD for its diffraction grating[11] or those that use diode lasers as their excitation light source[10, 19-21]) to the more sophisticated (e.g., those that use electronic circuits with an LED as their excitation light source[12, 13, 22, 23]) to the rather complex (e.g., those built using a nitrogen laser[18] or the parts from two working spectrophotometers[17]). Still other designs employ smartphones or combine digital cameras with electronic tablets or other computers to estimate the fluorophore concentration of different solutions.[14, 15, 24] In addition, one recent model uses 3D-printed parts, LEDs, and other circuit components to construct such an instrument.[25]

In the same spirit, we have designed two relatively inexpensive, single-beam, filter fluorometers to use as educational tools. The first model can be assembled from four 3D-printed parts (Fig. 2A); and, for those who do not have a 3D-printer, the second version can be made from parts available at most hardware stores or online (Fig 2B). Importantly, like other inexpensive scientific instruments that we have created,[1-3] both designs are easy to understand and simple to use. They involve relatively few parts and require no advanced knowledge of (or specialized experience with) electronic circuits or soldering techniques (as may occur with some more sophisticated designs). Moreover, because the plans and materials needed to make these instruments (plus the underlying computer files) are all open source, we have named the design "the OPN Fluorometer," and we hope that these devices will help to expand the use of fluorometry (and the valuable lessons that can provide) in educational settings at all levels.

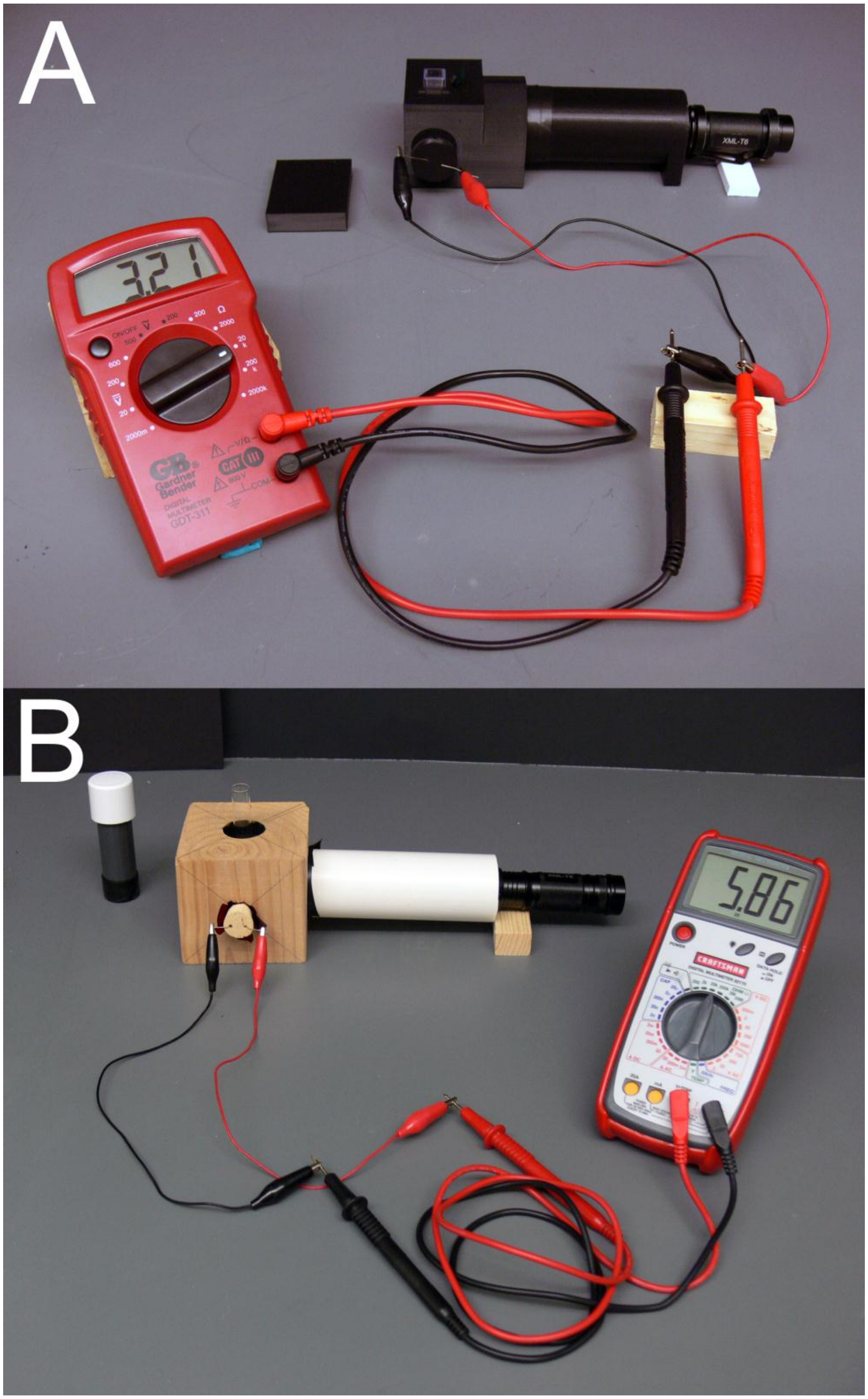


Figure 2. 3D-printed (A) and wooden (B) versions of the OPN Fluorometer.

MATERIALS AND METHODS

Similar to the OPN Colorimeter,[2] the 3D-printed version of the OPN Fluorometer has four components: (i) a body that holds a standard clear cuvette (or a small test tube) as well as cellophane excitation and emission filters, (ii) a lid that fits over the cuvette, (iii) a tube that holds the light source (a tactical LED flashlight), and (iv) a plug that fits into the side of the body and holds a detector – namely, a sensitive light dependent resistor or LDR (Fig. 3). As with other OPN instruments that we have developed,[1-3] we used the free version of DesignSpark Mechanical (RS Components, Corby, Northhamptonshite, U.K.) to design these parts. We have further included the underlying Computer Aided Design (CAD) files (S2) and related STereoLithographic (STL) files (S3) for these components as Supporting Information, so that readers can print or modify them based on their particular needs.

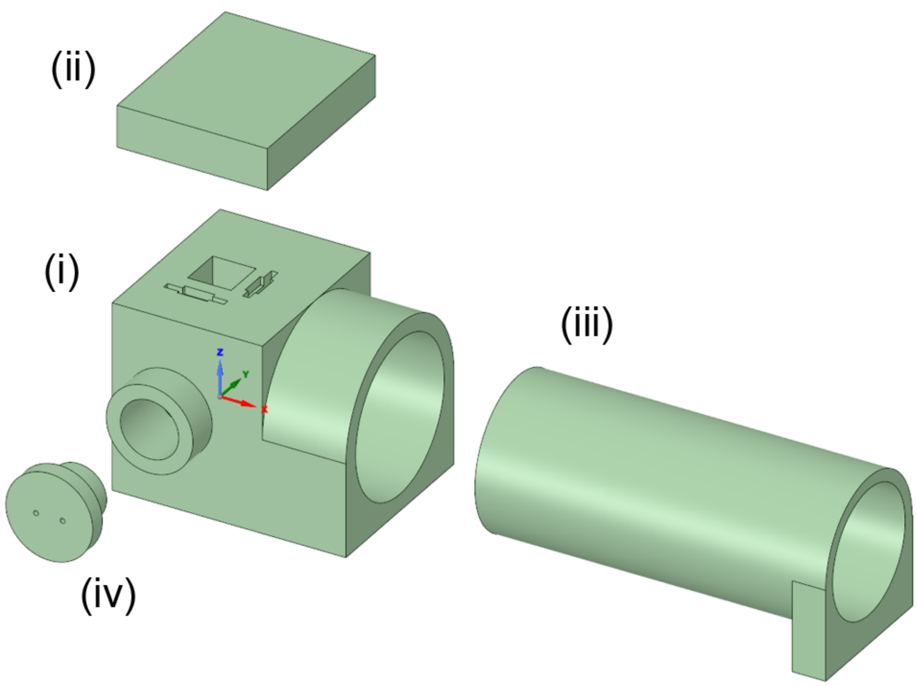


Figure 3. Schematic of the 3D-printed version of the OPN Fluorometer, which contains a (i) body, (ii) lid, (iii) tube for the light source, and (iv) plug that holds a sensitive light dependent resistor (LDR).

In addition, in the Supporting Information (S4), we provide instructions on how to build a wooden version of the OPN Fluorometer, which can hold a small test tube. To make this model, we simply drill holes of various sizes in the top, front, and side faces of a “4 x 4” block (Fig. 2B) to respectively hold (i) a ¾-inch PVC pipe and cap to cover the test tube; (ii) a 1½-inch PVC pipe for the flashlight; and (iii) a standard cork stopper for the LDR (S4).

In both versions of the OPN Fluorometer, we use a tactical LED flashlight that has a convex lens and adjustable head to focus the beam, which can generate a maximum brightness of 2,000 lumens (Fig. 2; S1 and S4). As with the OPN Colorimeter[2] and the “shoebox” fluorometer described above,[11] we also use small pieces of colored cellophane as our excitation and emission filters (S1 and S4; see Figure 4 below for the transmission spectra of these cellophane filters). As our detector, we use a sensitive LDR – specifically, an NSL-6910 photocell by Luna Optoelectronics) connected to a digital multimeter (S1, S4).

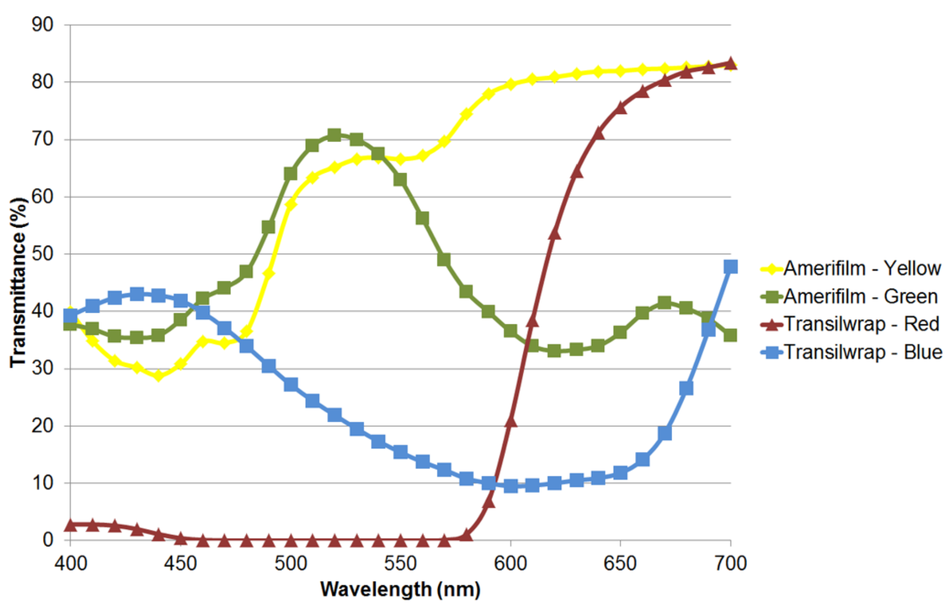


Figure 4. Transmission spectra for the Amerifilm and Transilwrap cellophane that we used as our excitation and emission filters in the 3D-printed and wooden versions of the OPN Fluorometer. Readers should note that this is the same figure contained in our OPN Colorimeter paper[2] since we used the same type of cellophane filters here as we did there.

We tested these designs by measuring the resistance values associated with increasing concentrations of two common fluorophores (Rhodamine B and Acridine Orange) in a standard glass cuvette or small Pyrex test tube (S5). At the conclusion of each assay, we then used the OPN Fluorometer to measure the resistance associated a known concentration of Rhodamine B or Acridine Orange, so that we could determine whether the previously collected data could be used to predict the concentration of an "unknown" solution. Once finished, we entered all of our data into a Microsoft Excel spreadsheet and displayed the results of our assays as scatter plots (Fig. 5). We then fit a curve to each set of data and displayed the equation for each curve (as well as its R^2 value) on the graph (Fig. 5).

Hazards

Readers should review the Supporting Information (S1, S4, and S5) for a discussion of the hazards associated with making the OPN Fluorometer and performing the fluorescence assays described above since, as explained in prior papers,[1-3] some of these dangers are significant (as would be expected in this type of context).

RESULTS

In the relative fluorescence assays that we conducted (S5), we consistently saw curves that changed dramatically (i.e., had steep slopes) as fluorophore concentration increased at low levels, but which began to taper off at much higher levels (Figs. 5 and 6). These general trends were consistent whether we used Rhodamine B (Fig. 5) or Acridine Orange (Fig. 6) in solution. However, because the resistance of the LDR that we used is inversely proportional to the intensity of the light striking it,[2, 3] the curves for both fluorophores appear to be "flipped" versions of the typical concentration curve that one would expect to see in this type experiment – i.e., the curves decrease (instead of increase) with concentration level (Figs. 5 and 6). Nonetheless, the trends in this data are similar to those appearing in other published reports[10] and generally comport with the type of curves that one would expect to see as the concentration of a fluorophore in solution increased.[15, 26]

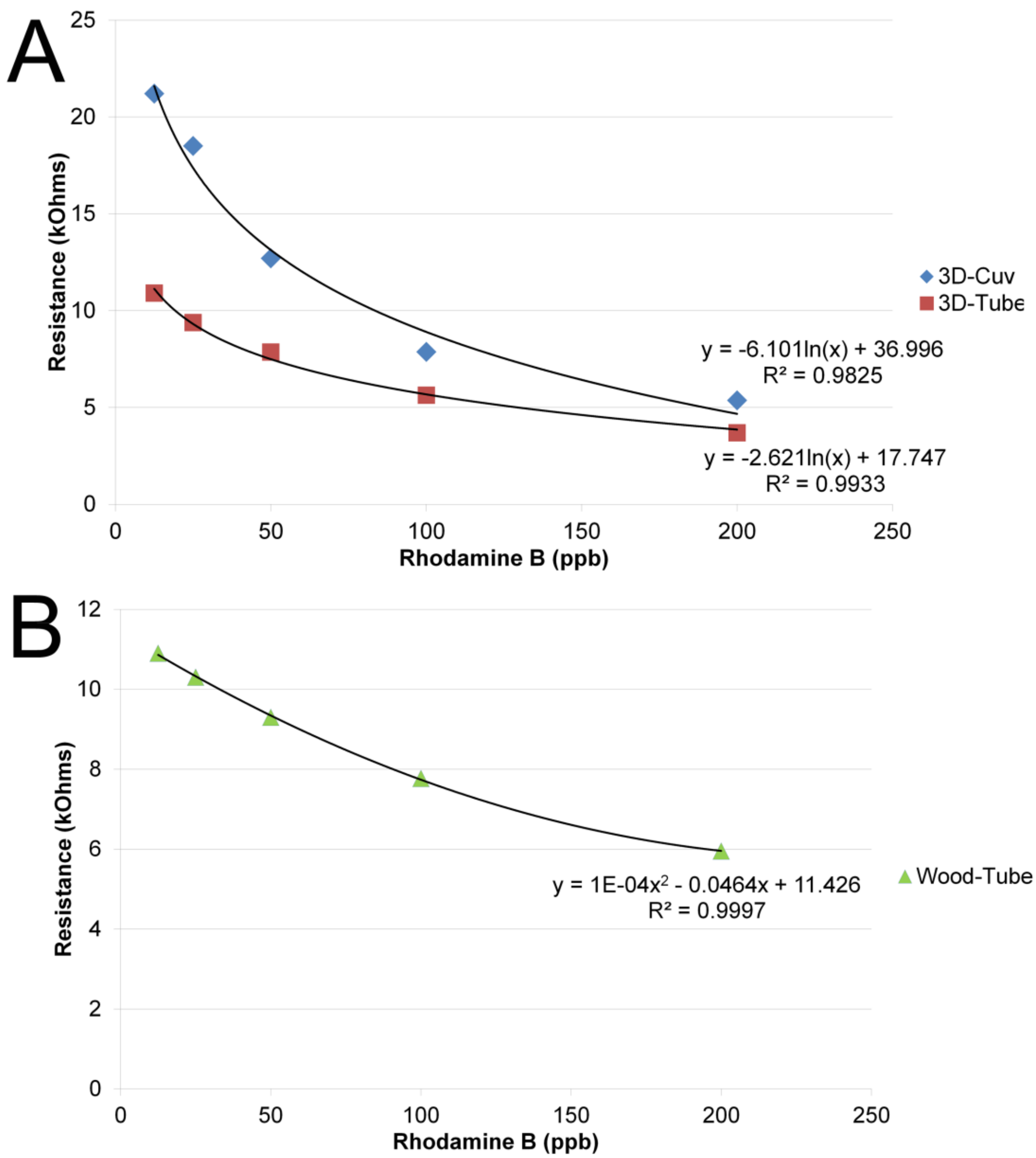


Figure 5. Representative results from our Rhodamine B assays using the 3D-printed (A) and wooden (B) versions of the OPN Fluorometer containing a glass cuvette (cuv) or test tube (tube).

We also found that well-established mathematical relationships consistently fit the data quite well, such as natural logarithmic ($y = a\ln(x) + b$), quadratic ($y = ax^2 + bx + c$), or power functions ($y = ax^b$) depending upon the exact set-up used (Figs. 5 and 6). In addition, in these experiments, the best-fit equations all had R^2 values over 0.97 (n = 18), and a majority of those (61%) had an R^2 value over 0.99 (n = 11).

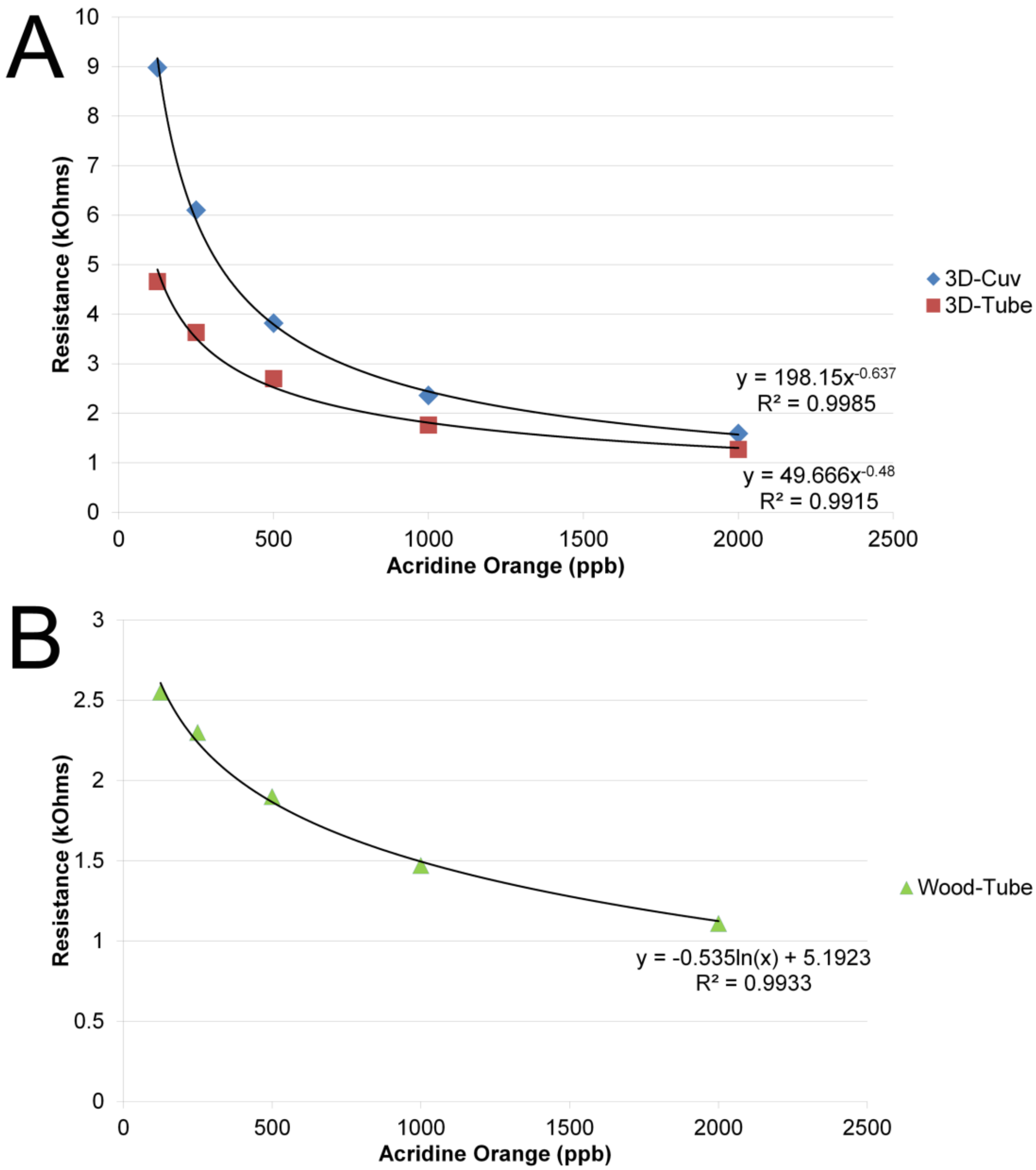


Figure 6. Representative results from our Acridine Orange assays using the 3D-printed (A) and wooden (B) versions of the OPN Fluorometer containing a glass cuvette (cuv) or test tube (tube).

Nevertheless, we typically did not see a linear relationship at the concentration levels used in our relative fluorescence assays, which began at 12.5 ppb for Rhodamine B and 125 ppb for Acridine Orange (S5). However, at lower concentration levels for both fluorophores, we were often able to obtain near linear trends in our results (S5), although a quadratic model frequently provided a perfect fit for the data ($R^2 = 1$).

Also, when it came to predicting the concentration of an "unknown" sample of Rhodamine B or Acridine Orange, we found that the best-fit curve associated with the data typically under-estimated the actual concentration level of the solution. In particular, in our Rhodamine B tests, we found that these best-fit equations under-estimated the true concentration level by 14.1% on average with a standard deviation of 18.4% ($n = 7$). Similarly, in our Acridine Orange tests, we found that the best-fit equations under-estimated the true concentration level by 9.89% on average with a standard deviation of 31.0% ($n = 9$). We believe that these discrepancies are likely due to several combined factors (S5), which typically cause the resistance of the LDR to "drift" upwards over time (e.g., a dimmer light beam caused by a draining battery, dimmer fluorophores caused by any bleaching or quenching in the solution, etc.). Thus, in our experience, a longer delay in measuring the resistance associated with an "unknown" sample typically resulted in a higher degree of error in absolute terms, although this was not always the case (S5).

DISCUSSION

As with the other OPN instruments that we have developed,[1-3] we designed the OPN Fluorometer to be easy to understand and simple to use. For example, like the OPN Colorimeter,[2] there are only four parts to the OPN Fluorometer, and these components fit together intuitively. As a result, students can easily take apart the instrument and look inside of it to gain a deeper understanding of how the device (and, thus, its more sophisticated counterparts) actually work – an extremely useful insight, as others have observed.[9-11, 13-15]

In addition, because the OPN Fluorometer (like the OPN Colorimeter and OPN Spec) uses an LED flashlight along with a commercial multimeter and LDR,[2, 3] students (and their teachers) do not need to be familiar with electronic circuits or soldering techniques in order to assemble (or work with) the instrument. Consequently, the device can be easily understood and used by students at many different levels.

Plus, because students can use different colors of cellophane for their filters in the OPN Fluorometer, the device is not limited to a single wavelength (as may occur with some LED-based designs or those that use laser points).[2, 3, 11] Instead, given the colors of various cellophane sheets or rolls that are available (S1, S4), the OPN Fluorometer can cover the entire visible spectrum (400 to 700 nm), which would enable students to examine the properties of many different fluorophores in solution, such as Coumarin 152 dissolved in methanol (ex: ~400 nm; em: ~435 nm);[21] fluorescein (ex: ~460 nm; em: ~495 nm);[13, 15] and Nile Blue A dissolved in methanol (ex: ~625 nm; em: ~665 nm).[20]

Moreover, a number of manufacturers now make tactical LED flashlights with UV light sources in them, and several of these models are available on websites like Amazon[27] for around $10 or less (at November 2016 prices, not including any taxes or shipping). As a result, instructors could use these UV flashlights (without an excitation filter present) to conduct additional fluorescence experiments, such as those involving: fluorescent whitening agents (ex: 340 to 370 nm; em: 420 to 470 nm);[23] quinine (ex: 250 and 350 nm; em: 450 to 455 nm);[10, 28-30] and thiamine or Vitamin B1 (ex: 365 nm; em: 444 nm).[31] However, in these instances, students should not use a glass or clear plastic (i.e., polystyrene or polyethylene) cuvette or test tube in the OPN Fluorometer since those materials tend to absorb UV light.[7] Instead, in these instances, students would need to use a quartz or silica cuvette or test tube in order to ensure that the appropriate wavelengths were reaching the solution.[8] Teachers and students should also make sure to wear the proper protective equipment during these experiments (including eye and skin protection) given the dangers posed by UV light.

Thus, given its versatility, the OPN Fluorometer should enable teachers to create a wide variety of classroom demonstrations or teaching lab activities. For example, in a chemistry, biology, or physics course, an instructor could use the OPN Fluorometer to demonstrate some of the basic principles underlying fluorescence spectroscopy or filter fluorometry, such as how fluorometers are designed and built, how they generate their results, and what that data means given the specimen(s) analyzed. In particular, by using the OPN Fluorometer, students could see that, in fluorometry, they are examining the light emitted from the molecules themselves (and not the light source), which is why the measurements are taken at a 90° angle to the light beam.[11] Students could also observe the Stoke's shift – namely, how the emitted light occurs at a longer wavelength (and, thus, a lower energy level) than the light used to excite electrons in the sample.[10, 11, 14] Students could also witness firsthand how the intensity of fluoresced light increases with the concentration of fluorophores in solution.

In addition, as described above, students could use the OPN Fluorometer to create basic calibration curves for a variety of different fluorophores (Figs. 5 and 6). Teachers could further demonstrate how these types of standard curves are used to estimate the concentration of an "unknown" by choosing a sample point or two on the curves for each lab group and telling the students to imagine that they obtained those resistance values for their unknown samples (S5). Students could then use those resistance values and the corresponding equation for their curve to estimate the concentration of their hypothetical unknown (S5). Alternatively, instructors could have their students actually measure the resistance associated with a physical sample. However, given the issue of "drift" described above, students may find that their estimates are significantly different than the "true" value, which might lead to some confusion or frustration amongst the lab groups (especially in an introductory class). Nevertheless, instructors could take this opportunity to teach their students about how more advanced equipment controls for this type of drift, which might be a useful way to introduce students to the intricacies of more sophisticated scientific instruments.

Besides generating basic calibration curves, however, the OPN Fluorometer could also be used to conduct other educational or instructional experiments, such as those demonstrating the phenomenon of fluorescence quenching (i.e., the reduction in fluorescence intensity due to various chemical and physical phenomena in solution)[25] and how quenching can be modeled using the Stern-Volmer equation,[9, 10, 15, 25, 32] the effects of oxidative degradation on a fluorophore like Nile Blue A,[20] or the generation of fluorometric titration curves using substances like Quinine whose fluorescence is pH-dependent.[25] Plus, students could use the OPN Fluorometer to conduct various enzyme assays (and then analyze the kinetics of these reactions), explore the intrinsic fluorescence exhibited by different protein structures in the presence of cofactors like tryptophan and FAD, or examine changes in membrane structure given changes in the surrounding environment (e.g., temperature or ion concentration).[6] As a result, the instrument should hopefully enable teachers to create a variety of educational and instructional activities for their teaching labs or in-class demonstrations.

Finally, with respect to cost, for readers who do not have access to 3D printing technology, the parts of a single OPN Fluorometer could be printed on a website like MakeXYZ[33] for roughly $67 at November 2016 prices, not including any taxes or shipping (S1). Thus, with a reasonably priced 2,000-lumens tactical LED flashlight ($15), a sensitive LDR ($8), a standard multimeter ($17), and a new 4x objective lens ($15), instructors could create a single set-up for around $122 (or less if they had access to a 3D printer). Specifically, for schools that already have a 3D printer, we estimate that it would cost between $80 and $100 to buy the four spools of filament needed to make the parts for at least a dozen OPN Fluorometers at November 2016 prices, not including any taxes or shipping (S1). Thus, with the additional equipment described above, a school could outfit a teaching lab with at least 12 such instruments for under $800 (or roughly $65 per OPN Fluorometer). Similarly, buying the raw materials to make a wooden version of the OPN Fluorometer should generally cost between $52 and $112 depending on the exact materials used (S4), not including the price of tools.

Consequently, a classroom or teaching lab could easily be equipped with several working OPN Fluorometers at a relatively low cost, which should create a variety of educational and instructional opportunities for teachers and their students. As such, we hope that the OPN Fluorometer will be both an affordable and useful tool for those in many different educational settings.

CONCLUSION

As we seek to develop more ways to make science more accessible and understandable for others, we hope that the OPN Fluorometer will help to foster scientific exploration in schools at all levels. We further invite readers to use or modify these designs to fit their particular educational needs, and we hope to one day see the results.

ASSOCIATED CONTENT

Supporting Information

The following materials are available on our website at http://pages.stolaf.edu/opn-lab/equipment/: our protocols for 3D printing the parts for the OPN Fluorometer (S1), the underlying CAD and STL files for the components of the 3D-printed version of the OPN Fluorometer (S2 and S3, respectively), instructions for building a wooden version of the OPN Fluorometer (S4), and our protocols for the fluorescence assays described above (S5).

AUTHOR INFORMATION

Corresponding Author

* Email: giannini@stolaf.edu

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Stewart, C.; Giannini, J., Inexpensive, open source epifluorescence microscopes. *Journal of Chemical Education* **2016,** 93, (7), 1310-1315.

(2) Stewart, C.; Giannini, J. Inexpensive, open source colorimeters that are easy to make and use. http://pages.stolaf.edu/opn-lab/equipment/ (accessed Nov. 2016).

(3) Stewart, C.; Giannini, J. Insexpensive, open source spectrophotometers that use part of a CD or DVD as a diffraction grating. http://pages.stolaf.edu/opn-lab/equipment/ (accessed Nov. 2016),

(4) OPN Lab Equipment Web Page. http://pages.stolaf.edu/opn-lab/equipment/ (accessed Nov. 2016),

(5) Freifelder, D., *Physical Biochemistry: Applications to Biochemistry and Molecular Biology*. Second ed.; W.H. Freeman and Company: New York, NY, 1982; pp 537-547.

(6) Gordon, D. B., Section 12.5: Spectrofluorimetry. In *Principles and Techniques of Biolchemistry and Molecular Biology*, 6th ed.; Wilson, K.; Walker, J., Eds. Cambridge Univeristy Press: New York, NY, 2005; pp 571-579.

(7) Lancas, F. M.; Carrilho, E., Molecular Fluroescence and Phosphorescence. In *Ewing's Analytical Instrumentation Handbook*, 3rd ed.; Cazes, J., Ed. Marcel Dekker: New York, NY, 2005; pp 141-161.

(8) Skoog, D. A.; Holler, F. J.; Nieman, T. A., *Principles of Instrumental Analysis*. Fifth ed.; Harcourt Brace College Publishers: Philadelphia, PA, 1998; pp 355-371.

(9) Bigger, S. W.; Ghiggino, K. P.; Meilak, G. A.; Verity, B., Illustration of the Principles of Fluorometry - an Apparatus and Experiments Specially Designed for the Teaching Laboratory. *Journal of Chemical Education* **1992,** 69, (8), 675-677.

(10) Delorenzi, N. J.; Araujo, C.; Palozzolo, G.; Gatti, C. A., A simple device to demonstrate the principles of fluorometry. *Journal of Chemical Education* **1999,** 76, (9), 1265-1266.

(11) Wahab, M. F., Fluorescence spectroscopy in a shoebox. *Journal of Chemical Education* **2007,** 84, (8), 1308-1312.

(12) Yan, H. W., An inexpensive LED-based fluorometer used to study, a hairpin-based DNA nanomachine. *DNA Computing* **2005,** 3384, 399-409.

(13) Wigton, B. T.; Chohan, B. S.; Kreuter, R.; Sykes, D., The Characterization of an Easy-to-Operate Inexpensive Student-Built Fluorimeter. *Journal of Chemical Education* **2011,** 88, (8), 1188-1193.

(14) 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. *Journal of Chemical Education* **2015,** 92, (2), 310-316.

(15) Algar, W. R.; Massey, M.; Krull, U. J., Assembly of a Modular Fluorimeter and Associated Software: Using LabVIEW in an Advanced Undergraduate Analytical Chemistry Laboratory. *Journal of Chemical Education* **2009,** 86, (1), 68-71.

(16) Chen, G. Y., Versatile portable fluorometer for time-resolved luminescence analysis. *Review of Scientific Instruments* **2005,** 76, (6).

(17) Hadley, F. J.; Mahloudji, A., A Home-Built Spectrofluorometer. *Journal of Chemical Education* **1990,** 67, (9), 806-807.

(18) Jones, B. T.; Smith, B. W.; Leong, M. B.; Mignardi, M. A.; Winefordner, J. D., A

Simple, Portable Fluorimeter with a Compact, Inexpensive Nitrogen Laser Source. *Journal of Chemical Education* **1989,** 66, (4), 357-358.

(19) Tellinghuisen, J., Laser-induced fluorescence in gaseous I-2 excited with a green laser pointer. *Journal of Chemical Education* **2007,** 84, (2), 336-341.

(20) Tran, Y.; Whitten, J. E., Using a diode laser for laser-induced fluorescence. *Journal of Chemical Education* **2001,** 78, (8), 1093-1095.

(21) Whitten, J. E., Blue diode lasers: New opportunities in chemical education. *Journal of Chemical Education* **2001,** 78, (8), 1096-1100.

(22) Wickliff, J. L.; Wickliff, D. E., Instrumentation for Measuring Invivo Chlorophyll Fluorescence Induction. *Journal of Chemical Education* **1991,** 68, (11), 963-965.

(23) 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. *Journal of Chemical Education* **2011,** 88, (8), 1182-1187.

(24) Yu, H. J.; Tan, Y. F.; Cunningham, B. T., Smartphone Fluorescence Spectroscopy. *Analytical Chemistry* **2014,** 86, (17), 8805-8813.

(25) 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. *Journal of Chemical Education* **2016,** __, (__), __-__ (available at http://pubs.acs.org/doi/abs/10.1021/acs.jchemed.6b00495).

(26) Ryan, M. A.; Ingle, J. D., Fluorometric Reaction-Rate Method for the Determination of Thiamine. *Analytical Chemistry* **1980,** 52, (13), 2177-2184.

(27) Amazon Home Page. https://www.amazon.com/ (accessed Nov. 2016),

(28) Chen, R. F., Some Characteristics of Fluorescence of Quinine. *Analytical Biochemistry* **1967,** 19, (2), 374-&.

(29) O'Reilly, J. E., Fluorescence Experiments with Quinine. *Journal of Chemical Education* **1975,** 52, (9), 610-612.

(30) O'Reilly, J. E., Interpretative Experiments - Quinine Fluorescence-Spectra - Dry-Lab Spectral Analysis Experiment. *Journal of Chemical Education* **1976,** 53, (3), 191-193.

(31) Bower, N. W., Kinetic Fluorescence Experiment for the Determination of Thiamine. *Journal of Chemical Education* **1982,** 59, (11), 975-977.

(32) Cumberbatch, T.; Hanley, Q. S., Quantitative imaging in the laboratory: Fast kinetics and fluorescence quenching. *Journal of Chemical Education* **2007,** 84, (8), 1319-1322.

(33) MakeXYZ home page. https://www.makexyz.com/ (accessed Nov. 2016),