Inexpensive, Open Source Colorimeters That Are Easy to Make and Use

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ABSTRACT

As part of an effort to help reduce the cost of scientific equipment and make scientific inquiry more accessible and understandable for others, we describe two different open source designs for inexpensive, single-beam colorimeters that are easy to build and use. One can be 3D printed, and the other can be built from supplies available at most hardware stores or online. The former uses a light dependent resistor (LDR) and digital multimeter as a detector while the latter uses a commercial light meter. In both models, we use an LED flashlight with a convex lens to focus the beam as our light source and pieces of colored cellophane as our filters. We test these designs using the Bradford assay to measure protein concentration and a lysozyme assay to measure enzyme activity. We also explain how these instruments can be used in an introductory or upper level chemistry, biochemistry, or biology course to teach basic principles of colorimetry or spectroscopy, including how to create a standard calibration curve and examine enzyme kinetics. Finally, because we are making these designs open and accessible to all, we have named them "the OPN Colorimeter," and we further include as Supporting Information instructions on how to 3D print or build these instruments (along with the underlying computer design files), so that others can use or modify them to fit their educational or research needs.

ABSTRACT GRAPHIC

KEYWORDS

General Public, Analytical Chemistry, Biochemistry, Laboratory Instruction, Hands-On Learning / Manipulatives, Laboratory Equipment / Apparatus, Spectroscopy, Proteins / Peptides, Enzymes

INTRODUCTION

In a recent report,[1] we explained how to 3D print or build inexpensive epifluorescence microscopes for educational or other purposes. We called these instruments "OPN Scopes" because their plans and parts were designed to be open and accessible to all. We have further applied this approach to the development of other low-cost scientific instruments, and we describe here two ways to make inexpensive, single-beam colorimeters for use in the classroom or teaching lab.

A critical tool in many different branches of the sciences, a colorimeter can measure the concentration of a given solution by determining the amount of light absorbed by the mixture. Moreover, because the absorbance of a solution often changes as its chemical properties change, a researcher can use a colorimeter to obtain critical information about (and crucial insights into) a wide variety of experimental situations (such as creating a standard curve to determine the protein concentration of an unknown sample, examining the rate of an enzymatic reaction, or determining whether a chemical reaction has reached equilibrium). As such, this analytical instrument plays

an essential role in a diverse array of fields, ranging from biochemistry to materials science to medicine.

Similar to a spectrophotometer, a colorimeter works by measuring the difference between the intensity of incident light striking a sample (I) and the intensity of transmitted light passing through that sample and reaching a detector (T).[2-5] To make this determination, a colorimeter first sends an intense beam of light through an optical filter, which selects a particular wavelength from the beam and sends it into the sample (Fig. 1, left). That incident light then passes through the sample, and the resulting transmitted light ultimately reaches a detector, which calculates an Absorbance value (A) – namely, the proportion of light that the sample has absorbed (Fig. 2, right).

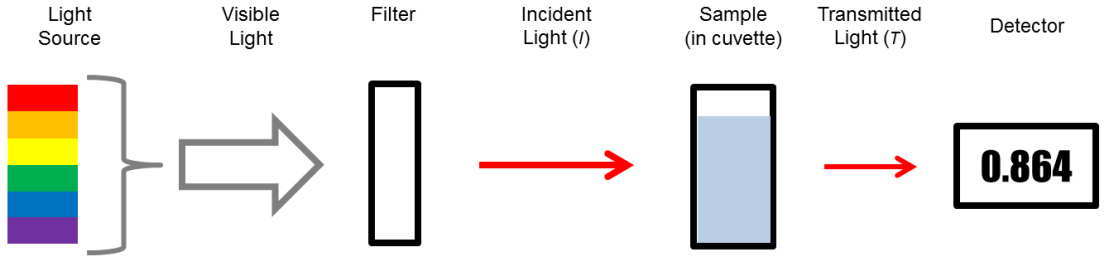


Figure 1. The workings of a basic colorimeter.

Moreover, as expressed in the Beer-Lambert law, there is a negative logarithmic relationship between the intensity of the light transmitted through the sample (T) compared to the intensity of the incident light initially striking the sample (I), and this relationship is further proportional to specific qualities of the sample. Namely,

$$A = -\log_{10}(T/I) = \varepsilon c l,$$

where ε is the molar extinction coefficient of the sample under investigation [$L/mol{\cdot}cm$], c is the concentration of that sample in solution [mol/L], and l is the path length of the sample [cm], which is typically the length of the cuvette containing the sample (i.e., 1 cm).[2-7]

Despite the importance of colorimetery and spectroscopy as an analytical and diagnostic tools, many commercial colorimeters and spectrophotometers are too costly to use in a teaching lab given the constrained budgets of many schools.[8-10] Furthermore, as others have noted, given the way in which many colorimeters and spectrophotometers are designed and used, students often view these devices as "black boxes" that give little insight into how they actually work.[11-14] In an effort to address these and other issues, many teams have offered some very insightful and innovative designs, giving students access to low-cost colorimeters and spectrophotomters that serve as valuable analytical and educational tools.[8-24] In fact, two recent models use 3D-printed parts, LEDs, and other circuit components to construct such an instrument,[25, 26] and another uses a smartphone as the detector.[27]

In a similar vein, we have designed two relatively inexpensive, single-beam colorimeters for use in the classroom or teaching lab. One version can be easily assembled from four 3D-printed components (Fig. 2A); and, for those who do not have access to this type of technology, the other model can be easily built from parts available at most hardware stores or online (Fig 2B). Moreover, both designs are simple to use and easy to understand, involving relatively few components and requiring no advanced knowledge of or experience with electrical circuits or soldering techniques (as sometimes occurs with more sophisticated designs). Because the instructions and supplies needed to make these instruments (as well as the underlying computer files) are all open source, we have named this general design "the OPN Colorimeter," and we hope that these devices can help expand the use of colorimetry in the classroom, teaching lab, and hopefully beyond.

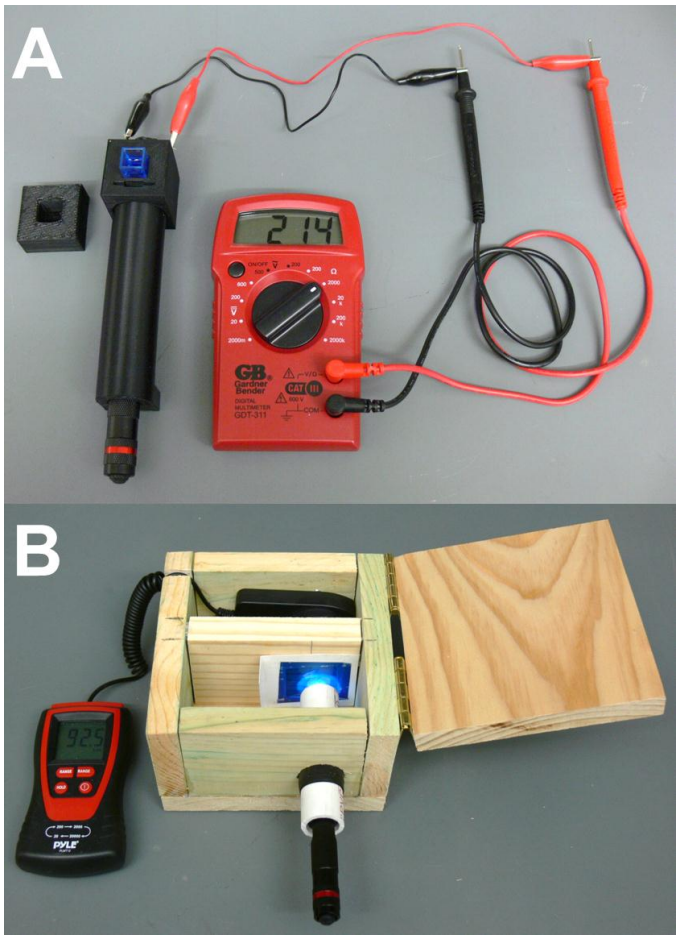


Figure 2. The OPN Colorimeter. (A) 3D-printed version. (B) Alternate version built from supplies available at most hardware stores or online.

MATERIALS AND METHODS

The 3D-printed version of the OPN Colorimeter consists of four parts: (i) a body that holds a standard cuvette and a cellophane filter, (ii) a lid that fits over the cuvette, (iii) a tube that holds the light source (an LED flashlight), and (iv) a plug that fits into the back of the body and holds a detector – specifically, a light dependent resistor (LDR) (Fig. 3). We have further included as Supporting Information the underlying Computer Aided Design (CAD) files (S2) and related STereoLithographic (STL) files (S3) for these parts, so that readers can print or modify them to fit their educational or research needs. As with the OPN Scope,[1] we used the free version of DesignSpark Mechanical (RS Components, Corby, Northhamptonshite, U.K.) to create these files (S1).

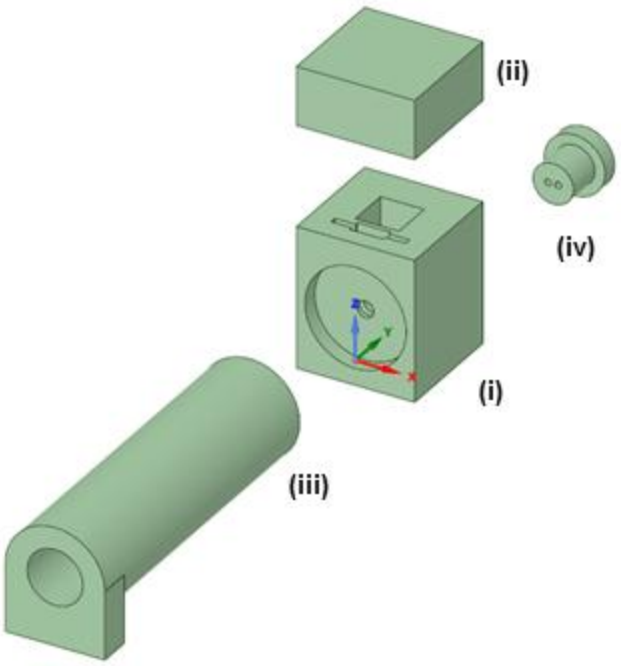


Figure 3. Schematic of the 3D-printed version of the OPN Colorimeter, which contains a (i) body, (ii) lid, (iii) tube for the light source, and (iv) plug that holds a light dependent resistor (LDR).

We also include as Supporting Information instructions on how to build an alternative version of the OPN Colorimeter using parts available at most hardware stores or online (S4). Thus, in brief, we built a wooden version of the OPN Colorimeter (Fig. 2B) using ¾-inch thick board, ¾-inch Schedule 40 PVC pipe, and other components, such as metal hinges and a wooden knob for opening and closing the lid (S4). Alternatively, readers can use PVC board instead of wood since the former should not warp, split, or rot over time.

Both versions of the OPN Colorimeter can hold a standard cuvette (external dimensions: 1.25 cm x 1.25 cm x 4.5 cm), and we further use a Coast G20 LED inspection light, which has a convex lens to focus the beam, as our light source in both models (Fig. 2; S1 and S4). However, other LED flashlights will suffice. We also use small pieces of colored cellophane as our filters (S1 and S4).

As our detectors, we use an inexpensive (85-cent) LDR and a digital multimeter with the 3D-printed version of the OPN Colorimeter (S1) and a commercial light meter with

the other version (S4). Of course, readers can also build a wooden or PVC version of the OPN Colorimeter that uses an LDR and multimeter, and we include some basic designs and assembly instructions for these models in the Supporting Information as well (S4).

We tested our designs using the Bradford assay[28] for determining protein concentration and an enzyme assay[29] for measuring the activity of lysozyme – both of which are described in the Supporting Information (S5 and S6). In addition, during these experiments, we used a commercial spectrophotometer (a Hitachi U-1100) as a control instrument.

After finishing each assay, we entered our data into Microsoft Excel and calculated an Absorbance value for each entry. Specifically, for the wooden version of the OPN Colorimeter, we used the formula $A = -\log_{10}(T / I)$ since the light meter reported its results directly in lumens per square meter [lux]. However, for the 3D-printed version of the OPN Colorimeter, which used a commercial multimeter with an LDR, we omitted the negative sign ("-") from this formula because the resistance of the LDR was inversely proportional to the intensity of the light striking it (instructors should emphasize this point with students to make sure that they understand the reason for this change). In both equations, I denotes the reading for an empty chamber and T denotes the amount of transmitted light for a given protein concentration (in the Bradford assay) or light scattering at a given time (in the lysozyme assay). Also, for both versions of the OPN Colorimeter, we treated the "dark" value as zero, which is the stated value for this variable in the commercial light meters that we used (and which did not impact the Absorbance calculations for readings taken with the LDR).

To generate our graphs, we subtracted the initial absorbance value from each reading to calculate the difference in absorbance for each assay, and we displayed our results as scatter plots using Excel (Fig. 4). Finally, we fit a line to the Bradford data

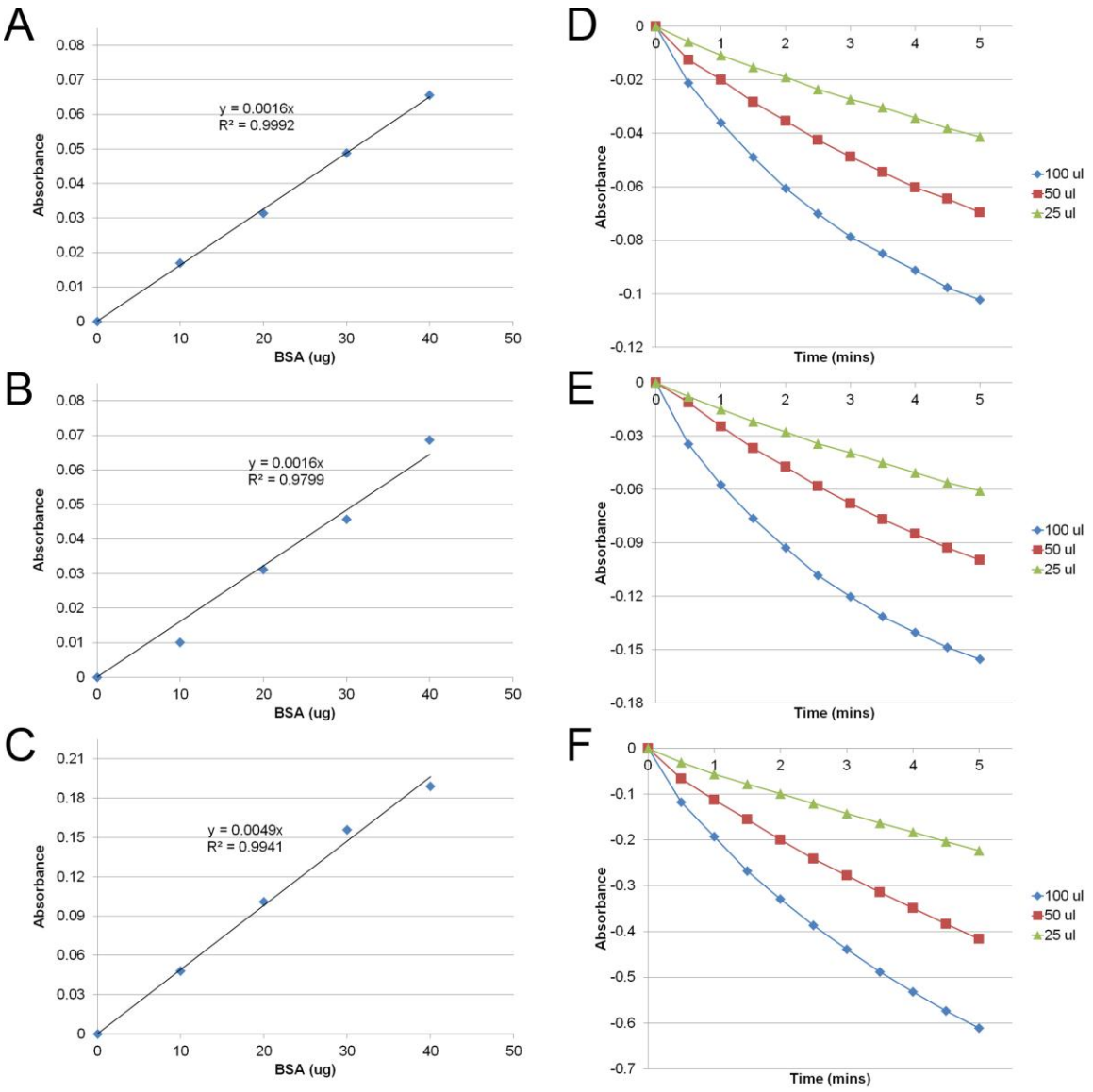
and displayed the equation for that line (as well as its R^2 value) on the graph (Figs. 4A – 4C).

Hazards

Before making the 3D-printed or wooden version of the OPN Colorimeter or performing any of the chemical assays described above, please review the Supporting Information (S1, S4 – S6) for a discussion of the related hazards since some of them are significant (as would be expected in any chemistry experiment or when working with mechanical tools).

RESULTS

In the two assays that we conducted, both versions of the OPN Colorimeter generated results that were similar to those of a commercial spectrophotometer (Figs. 4C and 4F) and consistent with the trends one would expect in these types of experiments.[28, 30] Specifically, in the Bradford assay, we found that absorbance increased linearly as the concentration of protein increased over the range that we used (Figs. 4A and 4B). Similarly, in the lysozyme assay, we saw different trends in the decay of each curve, which varied depending upon the concentration of the enzyme in the sample (Figs. 4D and 4E). In particular, as the amount of lysozyme in solution increased, the initial slope of the curve became steeper, and the curve itself began to taper off gradually over time (as typically occurs in enzyme-catalyzed reactions since the substrate is consumed more quickly at higher protein concentrations than at lower ones).[31, 32]



165 **Figure 4.** Results from a standard Bradford (left) and Lysozyme (right) assay using the 3D-printed (A and D) and wooden (B and E) versions of the OPN Colorimeter compared to those from a Hitachi U-1100 spectrophotometer (C and F).

Readers, however, should note that the curves pictured in Figures 4D – 4F are the mirror image of a typical saturation curve for an enzymatic reaction since the Absorbance of each sample decreased over time due to the breakdown of the bacterial

170 cell walls in solution. Readers should also note that, in both sets of assays, the OPN Colorimeter results were roughly $^1/_5$ to $^1/_2$ the magnitude of those generated by our control spectrophotometer – likely due to the broader spectrum of light that passes

through the cellophane filters that we used in the OPN Colorimeter (Fig. 5; see also S1 and S4) compared to the much more specific wavelength that would be transmitted by a commercial device.[33] As such, it is unlikely that students could use published molar extinction coefficients with the OPN Colorimeter. Nevertheless, as an exercise, students could calculate their own extinction coefficients given their data (e.g., $A = \varepsilon cl \rightarrow \varepsilon = {}^{A}/_{cl}$) and compare their results to published values.

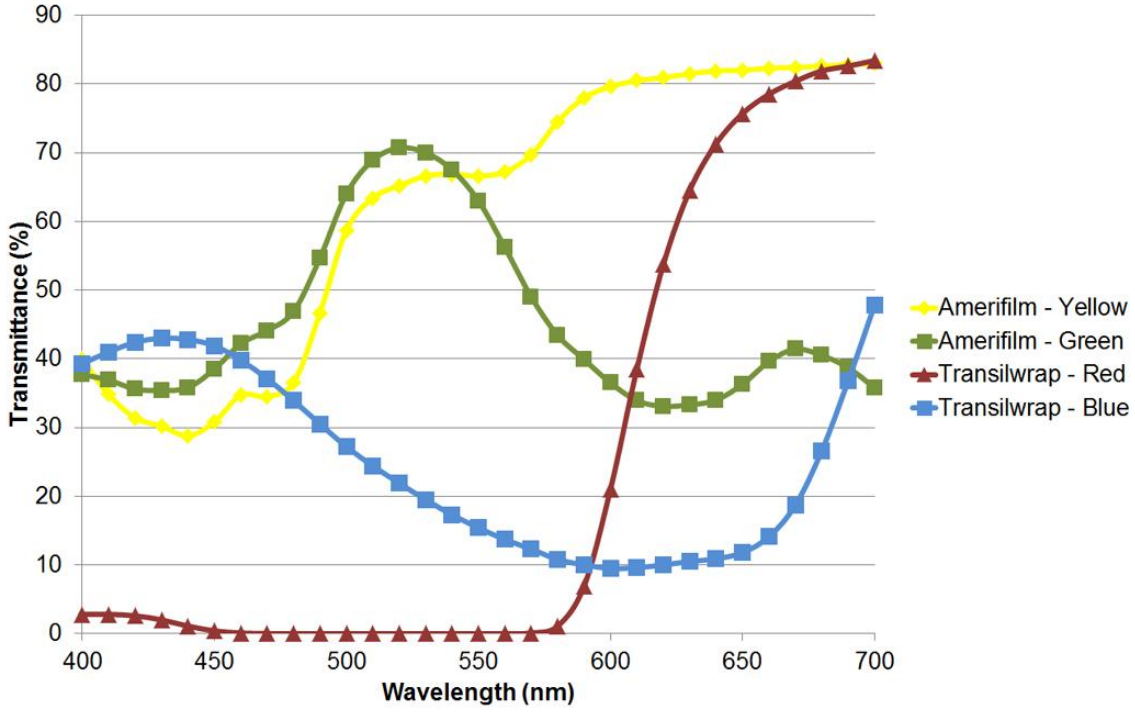


Figure 5. Transmission spectra (between 400 and 700 nm) for the Amerifilm and Transilwrap colored cellophane sheets that we have used as filters in the OPN Colorimeter (determined by using a Hitachi U-1100 spectrophotometer on the "VIS" setting).

DISCUSSION

As with the OPN Scope,[1] we designed the OPN Colorimeter to be simple and easy to make as well as use. Not only do the components fit together intuitively, students can easily take them apart and look inside to gain a better insight into how these (and, thus, more sophisticated) instruments actually work – a valuable quality, as others have noted.[9, 11, 13, 14] Furthermore, because the OPN Colorimeter uses a flashlight in

combination with a commercial multimeter or light meter, students (as well as their teachers) do not need to be familiar with electronic circuits or soldering techniques in order to assemble the instrument. In this respect, the device can be easily used and understood by students at almost any level. Moreover, because students can use cellophane filters of different colors in the OPN Colorimeter, the device is not limited to a single wavelength (as sometimes occurs with more sophisticated LED-based designs). As a result, even though the OPN Colorimeter is less sensitive than other models described in the literature given the wider spectrum of wavelengths that pass through these cellophane filters,[26] the instrument is rather versatile in addition to being relatively inexpensive to make.

In particular, as explained in the Supporting Information, we estimate that 3D printing the basic parts for the OPN Colorimeter using the MakeXYZ[34] or Shapeways[35] websites would cost between roughly $30 and $50 (S1) at April 2016 prices (not including any taxes or shipping). Thus, with a common LDR ($1) and a reasonably priced multimeter ($13) and LED flashlight ($11), the entire set-up should cost around $55 to $75 (or less for those who already have a 3D printer). Similarly, buying the raw materials to make a wooden or PVC version of the OPN Colorimeter should generally cost between $40 and $95 depending on the exact materials used (S4), not including the price of tools. As a result, a classroom or teaching lab could easily be outfitted with several working models at a fairly low cost, creating a host of opportunities for both students and their teachers.

For example, if used in an introductory or upper level chemistry, biology, or physics course, the OPN Colorimeter could help demonstrate many of the underlying principles involved in colorimetry and spectroscopy, including how these instruments are designed and built, how they obtain their readings, what those results actually mean given the sample(s) under investigation, and how those values factor into the Beer-Lambert law.

By way of illustration, students could not only use the OPN Colorimeter to create a standard (i.e., calibration) curve to determine the concentration of a given substance in a Bradford or similar assay; as part of that exercise, students could further use that curve to estimate the concentration of one or more "unknown" samples – a common component of many teaching labs. Similarly, in a lysozyme or other enzyme experiment, students could use the OPN Colorimeter to examine how the concentration of a given enzyme affects the shape of the decay or saturation curve as well as the initial rate of the reaction. Instructors could further ask their students to explain what their results imply for the solution they are examining (e.g., why a higher protein concentration results in a higher absorbance value in a Bradford assay or a steeper initial curve in a lysozyme assay). More importantly, though, instructors could expand upon these types of activities to provide even more challenging and engaging activities for their students.

For example, students could use the OPN Colorimeter to analyze a wide range of protein concentrations in a Bradford or similar assay, which would enable them to observe where the linear relationship expressed in the Beer-Lambert law begins to break down.[9] Additionally or alternatively, students could examine how different proteins (e.g., albumin, cytochrome c, gelatin, lysozyme, pepsin, or trypsin) react with Bradford reagent to generate different concentration curves,[36] how certain denaturing agents (e.g., detergents, such as Triton X-100 or SDS) unravel the protein and thus affect the Bradford results,[37] how various sugars (e.g., glucose or sucrose) interfere with the assay by sequestering the dye,[38] how other chemicals (e.g., Phenol) can be added to the assay to improve its sensitivity (depending upon the protein under analysis),[39] or how the Bradford assay can be improved upon by also examining absorption in the blue spectrum (450 nm) and using those results to linearize the data.[40]

Relatedly, students could use the OPN Colorimeter to conduct more sophisticated experiments into enzyme kinetics, such as exploring the effects of different pH levels, temperatures, inhibitors, or other denaturing agents on the underlying reaction.[29, 41] Students could also calculate (and then compare) the Michaelis-Menten kinetic parameters K_M, V_{max} , k_{cat}, and the catalytic efficiency of a reaction (k_{cat} / K_M) for different enzyme concentrations under different conditions and then explain what these results mean in the context of their experiment.

Moreover, given the wealth of lab activities and exercises published in the literature, the OPN Colorimeter could be used in wide variety of other experiments as well. For example, students could create calibration curves for different proteins, sugars, or chemical compounds (e.g., $CuSO_4$, $K_2Cr_2O_7$, or $KMnO_4$) through a Benedict, Lowry, or other assay.[8, 9, 12, 14, 20, 42, 43] Alternatively or additionally, students could examine the activity and kinetics of other enzymes, such as alpha-chymotrypsin,[44] amylase,[45] cytochrome-b5 reductase (methemoglobin),[46] or cytochrome oxidase.[47] In the process, we hope that students could use the OPN Colorimeter to not only gain a greater understanding of the underlying chemistry involved in these types of reactions, but also develop deeper insights into how scientific instruments like colorimeters and spectrophotometers are designed and used to collect data in these types of experiments. Thus, we hope that the OPN Colorimeter will be able to contribute to the outstanding and innovative work that others have already done (and are continuing to do) in developing low-cost scientific equipment for educational and other purposes.

CONCLUSION

As we continue to work on ways to help make science more accessible and understandable for others, we hope that both versions of the OPN Colorimeter will help to promote scientific inquiry and investigation in the classroom, teaching lab, and

hopefully beyond. We also invite others to use or modify these designs to fit their own educational or research needs, and we look forward to seeing the results.

ASSOCIATED CONTENT

Supporting Information

The following materials are available on our web page http://pages.stolaf.edu/opn-lab/equipment/: our protocols for 3D printing the parts for the OPN Colorimeter (S1), the underlying CAD and STL files for the components of the 3D-printed version of the OPN Colorimeter (S2 and S3, respectively), instructions for building a wooden or PVC version of the OPN Colorimeter (S4), and our protocols for the Bradford and lysozyme assays described above (S5 and S6).

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Notes

The authors declare no competing financial interest.

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