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A 3D-Printable Dual Beam Spectrophotometer with Multiplatform Smartphone Adaptor

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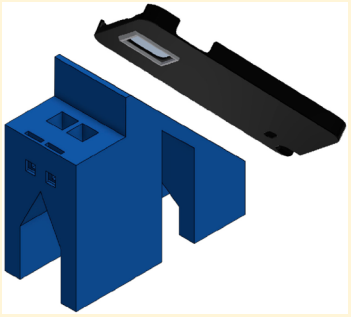
Supporting Information

ABSTRACT: Low cost, open-source analytical instrumentation has the potential to increase educational outcomes for students and enable large-scale citizen science projects. Many of these instruments rely on smartphones to collect the data, mainly because they can effectively leverage a dramatic price-to-performance ratio of the optical sensors. However, several hurdles need to be overcome for these devices to be more widely adapted. In this communication we focus on visible spectrophotometers, which are common in chemistry laboratories because of the day-to-day need for quantifying concentration. To make smartphone-based spectrometers practical for wider use, we have designed a 3D-printable spectrophotometer with a dual-beam optical geometry. This geometry allows for sample and reference data to be collected on the same photograph and thus improves the signal-to-noise ratio and reproducibility of the spectra. A universal mounting system was also developed to allow for a wide variety of smartphone form factors to be coupled to the spectrophotometer. To demonstrate potential applications of this device, two assays are reported. The first is a simple illustration of the Beer−Lambert Law with common household dyes. The second is a colorimetric nitrate assay, which shows a quantitative relationship between absorption and nitrate concentration. Kinetic data are also shown for the nitrate assay, which illustrate the long time-stability of the spectral data acquired from the device.



KEYWORDS: *Upper-Division Undergraduate, Physical Chemistry, Hands-On Learning/Manipulatives, Kinetics, Quantitative Analysis, UV−vis Spectroscopy*

INTRODUCTION

Accessibility to analytical instrumentation is a key hurdle to broadening participation in the chemical sciences and training a more diverse workforce.[1] The high cost of laboratory instruction limits accessibility, and thus opportunity, for students at institutions with small budgets. At institutions with sufficient funding, there is a learning paradox: modern, research-grade laboratory equipment is designed to keep the user from its working parts. Students can successfully make a measurement without ever getting a glimpse of the functional components, and therefore never gain an understanding of the core operational principles behind the digital interfaces.[2,3] Meaningful learning experiences are more likely to occur when students observe and interact with the working components of the instrument. This type of interactive learning creates a need for robust quantitative instruments that are low cost and yet are designed in a way that engages students and teaches them the core principles of the technique. In this communication, we describe an inexpensive dual-beam absorbance spectrometer, the DualSpec (Figure 1), which can be made with a 3D printer and less than US $2 in parts. The DualSpec can perform quantitative absorptivity experiments to teach the Beer−Lambert Law, as well as colorimetric enzyme kinetics assays.

Dual beam spectrophotometers became commercially available in the 1940s to improve signal-to-noise ratio and reproducibility in infrared and later UV−vis spectrometers.[4,5] This configuration, which utilizes a reference channel for comparison with that of the experimental sample, was especially effective at removing artifacts from fluctuations in the light source spectrum or intensity, the optical path, or the detector response. Modern instruments have been engineered to remove most of these artifacts, so the dual beam geometry is now used only in specialized research instruments. We propose that the recent wave of smartphone-based instruments has created a new potential application space for the dual-beam geometry.[6−14]

Smartphone cameras are optimized for image brightness and clarity, not necessarily for a linear intensity response.[6] Quantitative analysis, which assumes that pixel values are proportional to absolute intensity, is dubious due to complex and often proprietary dynamic range adjustments. In addition, low-cost light sources, such as incandescent bulbs or battery-powered LEDs, can have significant spectral shifts or intensity fluctuations. Controlling for these factors individually is possible, but it takes time and attention to detail, which presents a challenge in undergraduate laboratory courses where

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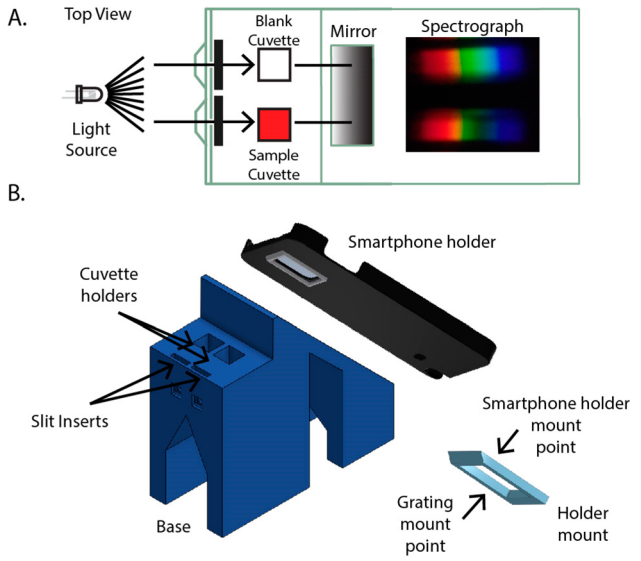

Figure 1. Overview of the DualSpec design. (A) A common light source enters two identical slits positioned 1.3 cm apart. Each slit creates a beam of light that passes through the cuvettes, is reflected vertically by a 2.54 cm square aluminum mirror and through a diffraction grating at the front of the rectangular holder mount. The spectra are recorded by the smartphone camera, and an example spectrograph is shown on the right of the schematic. (B) 3D CAD figure of the DualSpec parts. The base defines the light paths and holds the smartphone. The holder mount is glued to the diffraction grating on one side and a smartphone holder on the other side. The holder can be purchased or 3D-printed.

students of varying abilities are trying to finish experiments within a limited amount of time. Solving them in advance by instrument design would improve learning outcomes in the laboratory setting, as well as widen the accessibility of the instruments to citizen science projects.

We have engineered a dual beam geometry into a single 3D-printable device (Figure 1). The smartphone camera collects a sample spectrum and background spectrum on the same photograph, which significantly increases the signal-to-noise ratio and reproducibility of the data. This design is the first realization of a dual beam geometry for a 3D printable spectrophotometer, and enables new applications, including enzyme kinetics assays that will be described below. In addition to the dual beam optical path, we also addressed the need for the device to accommodate a wide variety of smartphone form factors. Previous do-it-yourself (DIY) spectrophotometers, including one from our lab, were built to work with a specific smartphone.[15] Adjusting the smartphone mount required changes to the 3D print file, which is impractical in teaching settings. Here we report a form-factor independent docking approach that allows the DualSpec to be used with any Android/iOS-based smartphone. The combination of a universal smartphone mount and dual beam optical path broadens both the potential student user base as well as the range of laboratory protocols that can be performed with the DualSpec. This approach could also be implemented in other low-cost DIY spectroscopy equipment that is under ongoing development.

RESULTS

Dual Beam and Phone Mount Design

We designed the DualSpec with two parallel light paths that are approximately 1 cm wide and are separated by a 3 mm barrier. This arrangement allowed the light in both paths to be reflected off a common 2.54 cm square mirror and into the diffraction grating and smartphone camera (Figure 1). Each path accommodated identical slits and cuvette holders. The light source was supplied by the user. For the data shown below, we used a desk lamp with an incandescent bulb. The lamp was placed some distance away from the entrance slits so that diffuse light entered the two slits at approximately equal intensity and was well below the saturation point of the camera. An example of the resulting spectrograph is shown in Figure 1A with a water sample in the top path and an absorbing solution in the bottom path.

We next developed a universal smartphone docking mechanism, in which a small rectangular adapter is attached to a smartphone holder (Figure 1B). The adapter serves two purposes. First, it is the mount for the diffraction grating film. Second, the adapter is the attachment point between the smartphone holder and the spectrometer base. These features make the DualSpec compatible with a wide range of modern smartphones. The 3D print files for the DualSpec are freely available online.[16] Protocol 1 in the Supporting Information section contains an itemized parts list, suggestions for 3D printing, as well as detailed assembly instructions.

Absorptivity Experiments

To test the DualSpec, we first collected serial dilution data in an assay first described in a chemical education report to teach the Beer–Lambert Law.[17,18] For this assay, a stock solution of a cherry flavored drink mix was made at 3.72 mg/mL in deionized water. The dominant food dye in this specific mix was FD&C Red No. 40.[18] The stock solution was diluted by a factor of 2, and each subsequent sample was a 1:2 dilution from the previous. The serial dilution was done six times, for seven total samples ranging from 3.72 mg/mL to 0.06 mg/mL. Deionized water was used as a blank. A 60 W desk lamp was used as the light source and was placed about 80 cm from the DualSpec device, with the slits facing the lamp. The smartphone used for data collection was an Apple iPhone6 using the standard iOS camera app in the default configuration. For each measurement, a photograph was taken with the sample in one cuvette position, and the buffer blank in the other. Photographs of the spectra were saved as JPEG files in 8-bit RGB format. The photos were converted into an 8-bit grayscale image using the MatLab function rgb2gray, which converts each RGB pixel value to a grayscale value by forming a weighted sum of the R, G, and B components: $0.2989 \cdot R + 0.5870 \cdot G + 0.1140 \cdot B$. The pixel intensity data were converted to two intensity spectra: $I_S(\lambda)$ for the sample path and $I_B(\lambda)$ for the blank path (Figure 2A). The sample and blank spectra were independently calibrated for wavelength using commercial fluorescent lighting as described previously.[15] Absorption spectra were calculated according to

$$A(\lambda) = -\log\left(\frac{I_S(\lambda)}{I_B(\lambda)}\right)$$

Despite the shifts in the light spectrum during the course of the experiment (Figure 2A, solid lines), no background subtraction or other corrections were necessary to obtain the absorption

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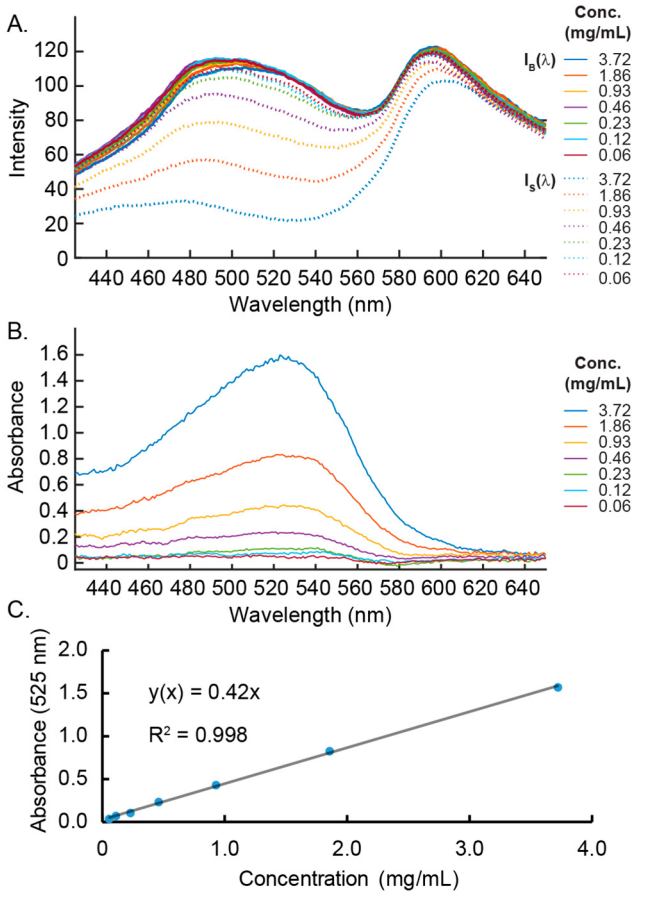

Figure 2. Visible absorption data for a serial dilution experiment. (A) Intensity spectra are calculated by summing pixel intensities at each wavelength. Solid lines indicate intensity spectra of the blank, $I_B(\lambda)$. Dashed lines are the intensity spectra of the samples, $I_S(\lambda)$, at the concentrations shown in the legend (3.72−0.06 mg/mL). (B) Absorption spectra, $A(\lambda)$, are calculated as described in the text without correction. (C) Absorbance vs concentration is plotted at the peak absorbance wavelength, 525 nm. The linear fit to the data gives $y(x) = 0.42x$ and is consistent with the Beer−Lambert Law: $A = \varepsilon lc$ ($R^2 = 0.998$).

spectra displayed in Figure 2B. The spectra have a maximum at 525 nm, and the intensity of the peaks at that wavelength was plotted against concentration to construct the Beer−Lambert plot (Figure 2C). The absorptivity was $\varepsilon = 0.42$ mL·mg^{-1}·cm^{-1}, which is similar to that reported on our original single-beam smartphone spectrophotometer (0.44 mL·mg^{-1}·cm^{-1}).[15]

To compare the quality of data obtained from the DualSpec to other spectrophotometers, we ran a linear regression analysis on the absorbance vs concentration data for the DualSpec, the SpecPhone, and a commercial spectrophotometer (Cary 100 UV−vis, Agilent Technologies). The percent error % ($100 \times \frac{\text{std. deviation}}{\text{fitted slope}}$) for the DualSpec data (Figure 2C) is 1.4%. The percent error for the same data collected on the SpecPhone is 5.3%, indicating a factor of four improvement of the DualSpec over the SpecPhone. As expected, the percent error for the same data collected on the commercial spectrometer is substantially smaller at 0.28%. These results indicate that the dual beam geometry improves the quality of the DualSpec to within an order of magnitude of a much more sophisticated and expensive commercial device.

Nitrate Concentration Assay

Many applications of visible absorption spectroscopy are concentration assays for a specific analyte in water. For molecules that do not have significant absorption at visible wavelengths, assays have been developed to either chemically modify the molecule or use it in a reaction that produces a new compound that absorbs visible light. For example, several colorimetric assays have been developed and commercialized for aquarium users to quantify the concentration of ammonia, chlorine, and various metal ions. Here we show that the DualSpec can be used to quantify the readout of these kits, using a variant of the nitrate reductase test,[19] which measures the concentration of NO_3^- in water from 0 to 60 ppm (mg/mL). For the hobby-store kit, readout is typically done by comparing the final reaction color to a card with indicator strips at calibrated levels. With an absorption spectrometer, however, it is possible to quantify concentration with higher accuracy and digitally record the data for reproducibility. Here we set out to define a set of experiments that can be performed on the DualSpec with the aquarium kit assay. A detailed protocol (Protocol 2) is provided in the Supporting Information section.

We first created a series of nitrate standard solutions by dissolving enough ammonium nitrate in Type 1 laboratory grade water to make a 100 ppm (100 mg/L) stock solution. Dilutions with were then made at 10, 20, 30, and 40 ppm. Aliquots of 5 mL from each of these solutions (as well as Type 1 water, 0 ppm) were used in the two-step assay as instructed by the commercial test kit (API, Chalfont PA). First, 10 drops of solution #1 were added to the sample and the mixture was inverted several times and allowed to rest for 2 min. During this time, the bottle of solution #2 was shaken vigorously for 30 s. Next, 10 drops of solution #2 were then added to the sample. The combination was shaken vigorously for 1 min and then rested for at least 15 min before measurements were made. The resulting solutions were then transferred to 1 cm cuvettes for the spectroscopy experiments (Figure 3A). Data were collected on the DualSpec with water in the cuvette in the blank position. Photographs were collected in triplicate with the default iOS camera settings for the iPhone 7 and processed as described previously. The resulting absorbance spectra are shown in Figure 3B. The water spectrum (gray) shows approximately zero absorbance across the wavelengths shown. The sample spectra show two peaks. The first peak at 440 nm is from the yellow reagent in solution #1 (seen in the 10 ppm solution in Figure 3A). Variations in the amplitude of the 440 nm peak are likely due to the manual addition of solution #1 by counting drops. The second peak at 545 nm is dependent on the nitrate concentration and has the effect of turning the sample progressively more red as the nitrate concentration increases. The 40 ppm solution blocks much of the light, and is thus noisier than the lower concentration spectra. The absorbance at 545 nm is plotted versus concentration in Figure 3C, which fits to a straight line with $y(x) = 0.072x - 0.005$ ($R_2 = 0.995$). The fit is consistent with the Beer−Lambert law, where $\varepsilon = 0.072$ ppm^{-1}·cm^{-1}.

Nitrate Assay Kinetics

In the previous sections we demonstrated that the DualSpec can measure the linear response of absorptivity with analyte concentration. In this section, we show that the improved optical geometry of the DualSpec enables the collection of time-dependent data and thus makes it amenable to enzyme

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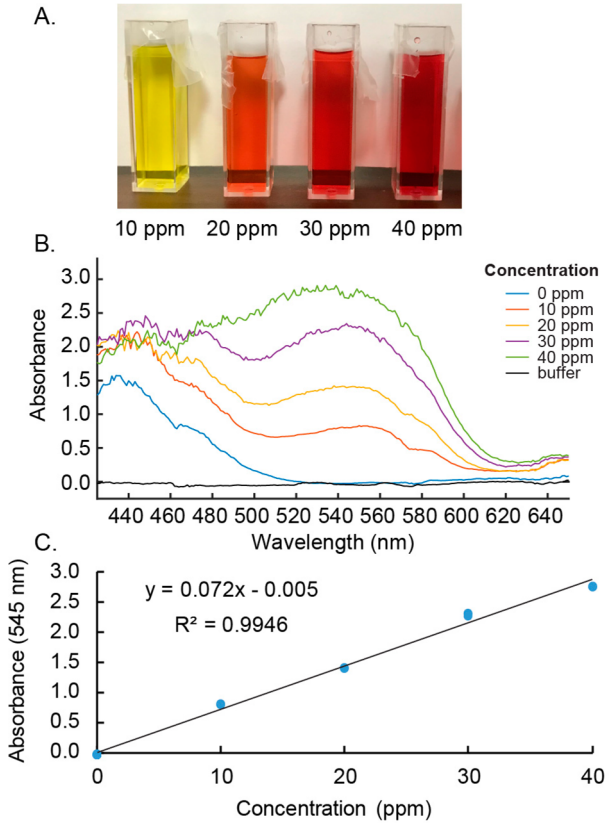

Figure 3. Aquarium nitrate concentration results. (A) Photograph of 10–40 ppm nitrate samples after running the assay described in the text. (B) Absorbance spectra for DI water (blank), DI water after running assay (0 ppm), and 10–40 ppm samples. Each absorbance spectrum was calculated with DI water in the reference cuvette. (C) Absorbance versus concentration at 545 nm is fit with a straight line and an unconstrained intercept $y(x) = 0.072x - 0.005$ ($R_2 = 0.995$).

kinetics assays. Such assays are part of upper division physical, analytical, and biological chemistry laboratory courses and several kinetic assays are reported in the chemical education literature.[20−23] Here, we demonstrate a simple application using the aquarium nitrate kit assay described in the preceding section.

To perform the experiment, a 30 ppm ammonium nitrate solution was prepared in Type 1 water. Solution #1 from the aquarium nitrate kit was added first. After the test tube is inverted five times, solution #2 is added. After a few seconds of shaking, a cuvette containing the mixture was placed in the DualSpec sample position, while Type 1 water was in the reference cuvette. After placing the sample cuvette, a video recording was started manually using the default iOS movie option in the camera application. Each frame from the resulting movie was analyzed in the same way as the photos above. This experiment was repeated several times and each showed the same characteristic time scales.

The data shown in Figure 4 are from a 5.5 min video recorded at the default 30 frames per second giving nearly 10,000 frames of data. A subsample of the calculated spectra (every 100th frame) is shown in Figure 4A. Overall, the quality of the data in a single frame is lower than when using the camera application (i.e., Figure 3B), likely due to down-sampling or compression algorithms applied during movie acquisition. The absorbance at 545 nm is calculated for all 10,000 frames and is plotted as a function of time in Figure 4B.

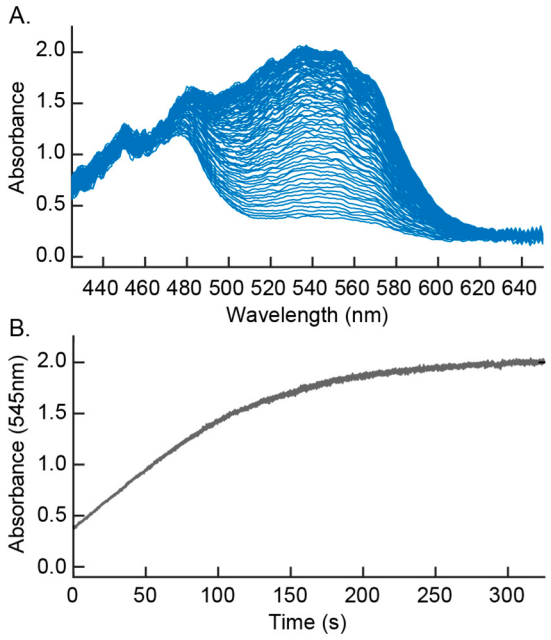

Figure 4. Kinetics of the nitrate assay. The nitrate reaction is initiated as described in text and then immediately placed in the sample position of the DualSpec. Data is collected in video mode and each frame is analyzed to produce an absorption spectrum. (A) Spectra are plotted for every 100th frame in the sequence. (B) Absorbance at 545 nm for every frame is plotted as a function of collection time.

Despite the noisier spectra, the overall stability of the data is high, yielding a remarkably smooth kinetic trace. The dead time of the experiment is several seconds because of the manual reaction initiation step, making quantitation of the trace difficult. However, the absorbance asymptotically approaches $A = 2.0$ at long times as expected from the calibration curve for a 30 ppm sample. The half-maximum of the reaction is about 50 s.

CONCLUSIONS

The central concept of absorbance spectroscopy is to relate the amount of light passing through a sample to the concentration of an absorbing species. This capability makes it useful in many areas of chemical research from materials science and catalysis to DNA quantitation and enzyme linked immunosorbent assays (ELISA). Absorbance spectroscopy or spectrophotometry is part of the curriculum for degree programs approved by the American Chemical Society. Several DIY photometers and colorimeters have been developed to provide structured and guided-inquiry approaches to the spectrophotometry experiments. A recent publication with student-assessment data suggests that this approach does in fact improve student learning.[24]

Here we designed a new low-cost spectrophotometer that retains key elements of commercial instruments with specific improvements aimed at increasing the range of applications both in terms of experiments and learning environments. Specifically, the dual beam optical path reduces the effect of sample to sample variability that can hinder quantitation of data from DIY instruments. Second, we designed a simple adapter that makes it amenable to data collection with a wide range of smartphone form factors. The combined features of the DualSpec will make it useful in laboratory teaching settings as well as in citizen science projects that make use of

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colorimetric assays. The dual beam geometry and the smartphone holder mount are likely to enable the development of new DIY spectroscopic instruments utilizing smartphones for data collection.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.8b00870.

Protocol 1. 3D print details, parts list (with suppliers) and assembly details (PDF, DOCX)

Protocol 2. Description of the nitrate assay in a way that can be implemented in a teaching laboratory (PDF, DOCX)

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Notes

The authors declare no competing financial interest.

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