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Teaching UV−Vis Spectroscopy with a 3D-Printable Smartphone Spectrophotometer

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Supporting Information

ABSTRACT: Visible absorbance spectroscopy is a widely used tool in chemical, biochemical, and medical laboratories. The theory and methods of absorbance spectroscopy are typically introduced in upper division undergraduate chemistry courses, but could be introduced earlier with the right curriculum and instrumentation. A major challenge in teaching spectroscopy is gaining access to laboratory equipment, which can be expensive. Even common educational spectrophotometers still carry a substantial cost and have the disadvantage of being inherently closed designs. We report on a 3D-printable smartphone spectrophotometer that is very inexpensive to build, yet retains the functionality and analytical accuracy necessary to teach concepts like the Beer−Lambert Law. The optical components are arranged in an intuitive, accessible way so that students can see each relevant part and experiment with the parameters. Here, we describe the device and provide exercises to teach different concepts in analytical spectrophotometry.

KEYWORDS: *Laboratory Instruction, High School/Introductory Chemistry, Upper-Division Undergraduate, Analytical Chemistry, Hands-On Learning/Manipulatives, UV-Vis Spectroscopy, Problem Solving/Decision Making, Calibration, Dyes/Pigments*

INTRODUCTION

Absorption spectroscopy is an experimental approach commonly used to measure the concentration of colored compounds in a sample. Because of a simple relationship between concentration and the amount of light absorbed, UV−vis spectroscopy has a wide array of applications in basic chemistry labs, biomedical research, and clinical diagnostics.

In biochemistry labs, for example, enzyme assays are often designed to track the presence of a colored compound downstream from the reaction in order to measure enzyme efficacy or the presence of a particular substrate. This is the basis of the enzyme-linked immunosorbent assay (ELISA), in which a colored compound is produced when an enzyme links to a targeted analyte.[1,2] The readout of an ELISA plate is an absorbance value of the colored product. Enzymatic reactions that lead to color changes of a solution have been translated to clinical settings where they are used to detect a wide range of targets.

Because of the ubiquity of absorbance spectroscopy in chemical, biochemical, and clinical settings, there is a clear need to educate students who will go on to careers in these areas. Basic spectroscopy methods are a core part of the undergraduate curriculum in chemistry departments and in degree programs like materials science, biology, and bioengineering. However, as a laboratory technique, it requires hands-on training and practice for a student to become proficient and knowledgeable in its use. This is difficult to achieve in one afternoon in a college lab; therefore, there is value in introducing the concepts and practices to students early and often during their education. This can be difficult and cost-prohibitive for many institutions.

Several low-cost, DIY (do-it-yourself) solutions to this problem have been developed in the past. Many of them are LED-based colorimeters, which relate absorption to concentration but do not directly output a spectrum.[3−10] This is fine for many applications, but it has drawbacks for teaching students how to read, understand, and quantify visible spectral features. Other devices are designed to display a visible spectrum, but are not ideal for making analytical chemistry measurements.[11−13] Several other device designs have been published that output a spectrum and have the basic components of a research-grade spectrometer to conduct analytical chemistry experiments.[14−18] These models have many working parts, making them better suited for long-term, special projects with small, focused groups of students.

Our approach was to design a compact DIY spectrophotometer that can be easily assembled and yet retains the ability to make accurate absorption measurements. By keeping the device simple, educators can design laboratory exercises that teach basic concepts such as spectral resolution and the Beer−Lambert Law. Our device is based on the cell-phone spectrometer reported by Alexander Scheeline.[19] In our experience, the main drawback of the Scheeline design was getting students to position the elements like the grating, cuvette, LED, and camera and to eliminate stray light. Cardboard tubes work nicely, but repeatedly positioning the

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cuvette and camera for accurate absorptivity measurements was not practical.

To solve this problem, we designed a 3D-printable housing around the optical path. This made it easy to position the parts and provided stability and light isolation. We call the device the SpecPhone, and have made the 3D print file publicly available.[20] The optical components are very inexpensive, and 3D printers are nearly ubiquitous at colleges, high schools, and public libraries. Online services are also available to print parts for very modest fees. This makes UV/vis technology available to a wider range of students, especially at institutions with limited funds. The SpecPhone also has several educational advantages over more expensive educational spectrometers, which are outlined below. This article will describe the spectrometer, the required parts, and the assembly process. We will also briefly describe the theory behind absorbance spectroscopy and provide laboratory exercises that can be done with the SpecPhone.

THEORY

An absorbance spectrophotometer compares the intensity of light across the visible spectrum in the presence and absence of a sample. In a generic configuration (Figure 1), a white light

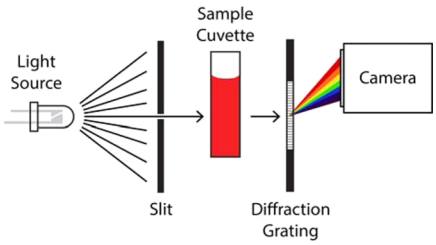

Figure 1. Light path and basic components of a visible spectrophotometer. The light source enters the spectrophotometer through a slit that limits the amount of light impinging on the sample in the cuvette. Light not absorbed by the sample then travels through a diffraction grating that splits the light into its component colors, much like a prism disperses white light into a color spectrum. The dispersed spectrum is then recorded by a detection camera.

source is sent through the sample and then dispersed onto a detector. The light passing through the device without the sample has a measured intensity, I_0, which is dependent on wavelength, $I_0(\lambda)$. With the sample in place, some of the light will be absorbed, and the resulting intensity of light at each wavelength is represented by $I_s(\lambda)$. Transmission at each wavelength is simply the ratio of these two quantities, and the absorbance, A, is defined as the negative base 10 logarithm of the transmission.

$$\text{Transmission} = T \equiv \frac{I_s(\lambda)}{I_0(\lambda)}$$

$$A \equiv -\log_{10}(T) = \log_{10}\left(\frac{I_0(\lambda)}{I_s(\lambda)}\right)$$

The Beer–Lambert Law tells us that absorbance is related to a parameter called the absorptivity, $\varepsilon(\lambda)$, multiplied by the length of the light path through the sample, l, multiplied by the concentration, c.

$$A = \varepsilon(\lambda)\cdot l\cdot c$$

Thus, if the absorptivity and path length are known, one can measure concentration from the absorbance. If the absorptivity is unknown, one can prepare several known concentrations to measure $\varepsilon(\lambda)$ experimentally.

DESIGN AND ASSEMBLY

A schematic of the SpecPhone is shown below in Figure 2. Central to the design is a support structure that will hold the

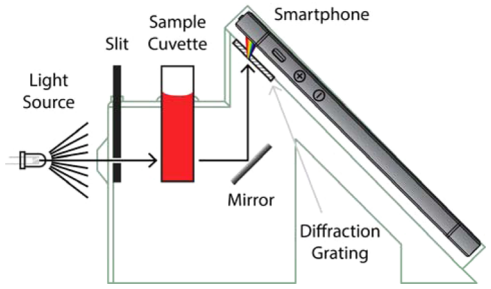

Figure 2. Schematic of the SpecPhone. A stable light source enters the SpecPhone apparatus and is spatially filtered by a removable slit that controls the geometry and intensity of light projected through the sample. The light transmitted through the sample is reflected off a mirror and through the diffraction grating placed at a 45° angle. This grating disperses the light into its color spectrum and into the smartphone camera, which can be viewed on the screen and saved as a photo.

slit, cuvette, grating, and camera in place so that spectral measurements can be made in a reproducible way. The drawing was made with SolidWorks software and exported as a 3D-printable file for production. Key elements of the design are listed below. Information about how to obtain a print file, further assembly details, and 3D print files are available at the author's Web site.[20] The print file is also available at the NIH 3D Print Exchange Web site.[21]

Light Source

The light source for the SpecPhone is provided by the user. It can be a desk lamp, flashlight, or LED. The only requirement is that it produces a stable light intensity and a relatively flat spectrum over visible wavelengths.

Slit

The slit was designed to be a removable part of the device. It is simply a rectangular slab with a horizontal opening (see Figure 3). The width of the opening is related to the resolution of the instrument: larger slit widths will smear out the component colors and reduce the ability to distinguish wavelengths while narrower slit widths will increase the sharpness of the spectral features. In our design, the slit can be removed and changed to illustrate the principle of spectral resolution.

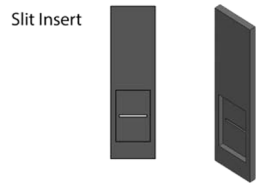

Figure 3. Removable slit insert. The 3D-printed removable slit insert is a 44 × 12 × 2.0 mm slab with a recommended slit width of 1.0 mm.

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Cuvette Holder

The cuvette holder in the SpecPhone is simply a rectangular orifice that allows for a 1 cm cuvette to be positioned in it. The 1 cm cuvettes are the industry standard and can be obtained at low cost from many suppliers.

Mirror

In the SpecPhone, the sample cuvette rests vertically as shown in Figure 2, requiring that the light path travel horizontally from the slit through the cuvette. The light is then reflected vertically so that a smartphone could be positioned at a comfortable 45° angle. The mirror is a 1 ×1 in.^{2} aluminum mirror available from many craft suppliers. It rests on a 45° internal face of the plastic support and is held into place with glue or double-sided tape.

Smartphone

For the smartphone, we first chose the iPhone 5 because of its popularity in the market, its high quality camera, and the potential for application development. The housing can be easily modified with 3D CAD software to accommodate other smartphone models.

Diffraction Grating

A transmission diffraction grating contains a series of parallel lines at a particular density (# lines/mm) that refract the light into the component colors much like a prism. The transmission efficiency and line spacing affect the quality and resolution of the spectrum. We chose an inexpensive grating that can be cut with scissors to fit the light path clearance. It is available as a 6 × 12 in.^{2} film sheet with 1000 lines/mm (Rainbow Symphony, Inc., Reseda, CA).

Assembly

After the housing is printed out, the mirror is installed using double-sided tape. The grating is then cut from a large film and positioned in the aperture near the camera port. The lines of the grating must be horizontal to the device so that light diffracts perpendicular to the slit. Once the grating and mirror are in place, the slit, cuvette, and camera can be positioned for measurements.

■ TEACHING APPROACH

Light and Wavelength

In this section, we outline a few simple exercises that can be done with students at various levels. The first example is to explore the dispersion of white light into its component colors. To demonstrate this with the SpecPhone, remove the cuvette and place a cover over the orifice to block extraneous light. With the iPhone camera app open, point the SpecPhone to various light sources in the classroom. For example, diffuse sunlight from a window will produce a rainbow image. Light from a computer monitor will often show three bands: red, green, and blue. Overhead fluorescent lighting will show well-defined lines corresponding to mercury and lanthanide luminescence. Examples of the spectral images are shown in Figure 4.

Converting an Image to a Spectrum

The SpecPhone is designed to teach basic principles of visible spectroscopy and related concepts such as the Beer−Lambert Law. To do this, the spectra must be analyzed quantitatively. In this activity, the goal is to teach students how to convert the photograph of the dispersed light into a spectrum that displays intensity versus wavelength. This procedure is an opportunity for students to learn about the connection between data collection and analysis. It is also a useful exercise to introduce students to basic data analysis tools in spreadsheet programs like Excel. To extract the pixel values from the image, we suggest using ImageJ, which is a free software developed by the National Institutes of Health.[22] It was created to examine medical imaging data and is now used for many image applications.[23] The program can process a range of file formats and perform a variety of functions that include calculating intensity, creating line profile plots, image transformations, and more. While it does represent a small learning curve, many students who go on to careers in medical and biological sciences will encounter this software later.

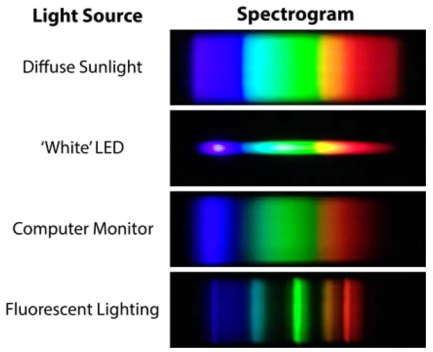

Figure 4. Visible spectra of different light sources. Each spectrogram is cropped from the full image recorded by the SpecPhone.

Once the spectral images have been taken with the SpecPhone, the photos need to be saved to the computer on which ImageJ will be used. One or more photos can be analyzed at a time, but we will describe the operations with one photo. After it is opened in ImageJ, the photo should be rotated approximately 90° so that the each vertical column of pixels corresponds to one wavelength region. Next, a selection box is drawn around the spectrum. In the vertical dimension, try to exclude as much dark area as possible from the box. In the horizontal dimension, leave ample space after the blue and red features so that the baseline can be identified clearly. Once the selection box is positioned, plot a profile of the selection. This sums the pixels in the vertical direction and plots the intensity versus pixel number in the horizontal dimension. The plot can be saved as an image or a text file (column of pixel, intensity values), or else the data can be copied directly into a spreadsheet program for further analysis. Figure 5 shows a cropped, rotated spectrogram with the corresponding spectrum below. This same set of operations can be performed on a stack of images collected during a set of experiments so that all the spectra have the same axis. Further details are available in Protocol 1 in the Supporting Information. The conversion between pixel position and wavelength will be described below.

Pixel Calibration

To calibrate the pixel positions to the corresponding wavelengths, a simple peak-fitting routine can be used. The first step is to measure a spectrum with sharp features of known wavelengths. One previously published approach is to use laser pointers,[17] which is also illustrated in Protocol 1 in the Supporting Information. Here, we outline a procedure that uses the spectrum of typical industrial fluorescent lighting (Figures 4 and 5). For the curious student, an assignment of these peaks is available on the Wikimedia Commons.[24] If a commercial, calibrated spectrometer is available, the spectrum of an identical light source should be measured with the SpecPhone and the

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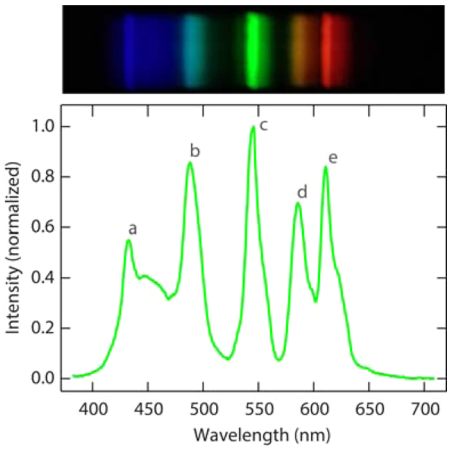

Figure 5. A SpecPhone photo (top) of a fluorescent light can be imported to ImageJ to plot intensity versus pixel position (bottom). Here, the pixel values have been converted to wavelength using the calibration protocol described in the text.

commercial spectrometer. In this way the peaks in each spectrum can be directly compared. An example of this comparison is shown in Table 1 below. Note that the relative peak intensities may not be identical in the SpecPhone and the commercial spectrometer due to differences in the detector sensitivities and the lighting manufacturer. To carry out the calibration, peak positions in wavelength are plotted against the corresponding peak positions in pixels, and the data is fit to a calibration function. The calibration function can be a higher order polynomial, but we opt for a linear function, which is consistent for the wavelength range here and is easy to calculate. The students should use Excel to scatter-plot the peaks on a wavelength versus pixel graph and then fit the data to a trend line. Alternatively, they can implement a linear regression analysis in the Excel data analysis toolbox. The resulting function is then used to convert each pixel position to a wavelength value. The *x*-axis in Figure 5 was calculated using this procedure.

Table 1. Peak Positions from a Calibrated Commercial Spectrometer and the SpecPhone

Peak[a]	Wavelength (nm)[b]	SpecPhone (pixel)[c]
a	434.5	1490
b	486.0	1316
c	544.0	1146
d	587.0	1017
e	611.5	943

[a]Labels correspond to those in Figure 5. [b]Peaks identified in spectrum measured by an AvaSpec HS1024x58TEC, with a 500 lines/mm grating and 50 μm slit (3.5−4 nm resolution). [c]Absolute pixel values are arbitrary and will correspond to the crop area.

Slit Width and Resolution

Resolution is a key variable in any spectroscopy experiment. The resolution of a spectrophotometer is the scale at which it can distinguish between different wavelengths of light. It can also be thought of as the width of a peak corresponding to a pure monochromatic source. The resolution of a spectrometer is limited by the properties of its components, including the diffraction grating, the detector, and the entrance slit. For example, increasing the line density of a diffraction grating increases the spectral resolution by more finely separating the component colors. The properties of the detector affect the resolution, mainly because of the size and density of the pixels. For a smart phone detector, the resolution is also limited by the lens optics preceding the detector.[17] The spectral density of the SpecPhone in the configuration described here is 0.33 nm/pixel, which is near the lower limit of the spectral resolution for the instrument.

The entrance slit directly influences the resolution by constraining the geometrical path of the light through the grating and detector. Theoretically, the slit could be narrowed to infinitely small values so that it does not affect the resolution, but at that point there would be no light entering the device. Consequently, there is a direct trade-off between spectral resolution and signal-to-noise when choosing a slit width. This is a fundamental property of a spectrometer, and it is important for students to understand the resolution principle and the factors influencing the choice of slit width.

To illustrate the role of the slit width in determining the instrument resolution, we designed the SpecPhone to have a removable slit, so that the effect could be observed directly. An example laboratory exercise is to measure the spectrum of fluorescent lighting for various slits widths. The 3D print files for several slits were created and printed out for this exercise. In Figure 6, the spectral range corresponding to peak c in Figure 5 is isolated to observe how that peak width changes with each slit. As shown in the bottom plot, the fwhm changes are consistent with the slit widths. Figure 6 also shows that there is essentially no improvement in resolution going from 0.6 to the 0.3 mm slit.

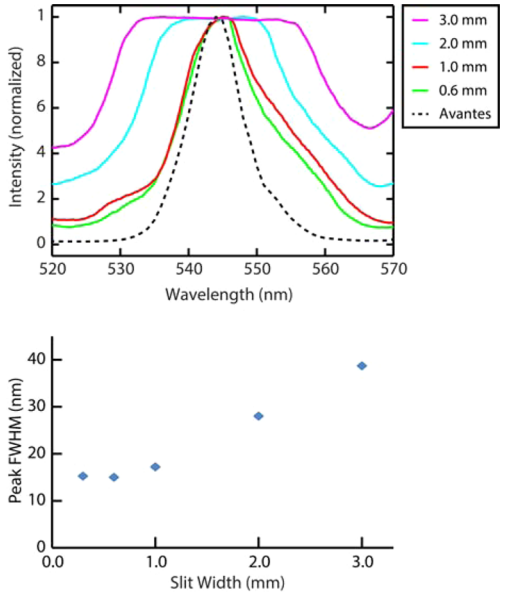

Figure 6. (Top) The intensity of a fluorescent light spectrum between wavelengths of 530 and 560 nm is displayed for different slit widths as indicated in legend. The dashed line corresponds to the spectrum measured with a commercial spectrophotometer (AvaSpec HS1024x58TEC, Avantes, Inc., calibrated resolution of 3.2−4 nm). (Bottom) The full-width, half-maximum (fwhm) of the peaks measured with the SpecPhone is plotted as a function of slit width. The peak width corresponding to the 0.3 mm slit (spectrum not shown) is nearly identical to that of the 0.6 mm slit, showing that narrower slits will not appreciably increase the resolution.

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The Beer–Lambert Law

The Beer–Lambert Law describes the relationship between the concentration of a particular analyte and its absorbance, A, and is defined by the equation:

$$A = \varepsilon(\lambda) \cdot l \cdot c$$

As described in the Introduction and Theory sections above, this relationship has a wide range of applications in laboratory and clinical settings. Here, we adapt a student laboratory protocol for use with the SpecPhone spectrophotometer.[25] The main goal of the exercise is to make an absorptivity calibration plot with several dilutions of a colored analyte. For demonstration purposes, we investigate the absorbance of a cherry flavored drink powder (i.e., Kool-Aid) because it is safe and inexpensive.[25] Other sample protocols have been published, which are compatible with the SpecPhone.[26−28]

The drink powder was purchased and a stock solution was prepared at 4 mg/mL in deionized water. Serial dilutions were prepared down to 0.0625 mg/mL (see Figure 7). Pure deionized water was used as the blank. A 60 W desk lamp was used as the light source, which was positioned about 0.5 m away from the spectrometer. The lamp intensity (or distance) needs to be adjusted so that the camera is not saturated at any wavelength. For each sample, the spectrum was measured with the cuvette positioned in the SpecPhone. The spectral photo was converted to an intensity spectrum, $I_s(\lambda)$, and the absorbance spectra were calculated with $I_0(\lambda)$, the spectrum of the blank (e.g., a cuvette with pure water).

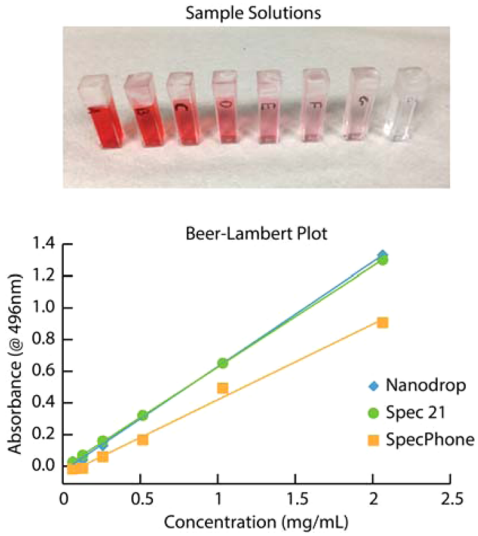

Figure 7. (Top) Photograph of sample dilutions. The stock concentration was 4.1 mg/mL (A) and each subsequent sample is a 1:2 dilution down to 0.64 mg/mL (G). (Bottom) The absorbance at 496 nm was measured by the SpecPhone and two commercial spectrometers. The absorbance is plotted as a function of concentration, and the fitted slope is a measure of the absorptivity, ε. The absorptivity values are 0.64 ± 0.01 for the Nanodrop, 0.629 ± 0.004 for the Spectronic 21, and 0.44 ± 0.02 for the SpecPhone.

The absorption spectrum has a clear maximum near 496 nm, and the intensity of the peak was plotted versus concentration (Figure 7). This is referred to as the Beer–Lambert plot. We found that for the stock solution there is not enough light passing through the sample for the iPhone camera to record a spectrum. For lower concentrations, the absorbance is linearly dependent on concentration from 0.0625 mg/mL to 2.0 mg/mL. This linear relationship is a clear demonstration of the Beer–Lambert law, and verifies that the SpecPhone can make analytically accurate measurements. For comparison, we measured the absorbance (λ = 496 nm) of each solution using two commercial spectrometers: Nanodrop 2000 (ThermoFisher Scientific), and the Spectronic 21 (Bausch and Lomb). The slope measured by the commercial instruments was consistent at 0.63, which is larger than that of the SpecPhone (0.44). This shift in the absorptivity was reproducible and is due to the sensitivity scaling in the iPhone camera.[17] The shift implies that the absorptivity of a specific analyte needs to be calibrated by the SpecPhone, rather than taken from literature or a handbook. A protocol outlining this procedure is included in the Supporting Information.

This simple exercise demonstrates that the SpecPhone can be used in a laboratory setting to measure the visible absorbance of a sample solution. After the absorptivity is calibrated with the Beer–Lambert plot, it is then possible to accurately determine the concentration of unknown samples of the same analyte. With this general approach, several types of laboratory experiments can be devised to teach the core principles of analytical spectrophotometry.

HAZARDS

No acute hazards are involved with the assembly and use of the SpecPhone. 3D printing involves minor hazards associated with the heat extrusion. The hazards associated with measuring absorbance are solely due to the analyte. In the case of the cherry drink powder experiment shown here, there are no health hazards, but standard laboratory safety guidelines should be followed. This includes personal protective equipment like safety glasses and gloves.

CONCLUSIONS

In this paper, we have reported on the SpecPhone, a 3D-printable smartphone spectrophotometer for chemical education. The device has been optimized to teach basic concepts in spectroscopy like the Beer–Lambert Law. The modular design encourages student interaction with the components, which helps develop intuition for the relationship between the device parameters and data collection. We have also included several exercises that teach basic principles of light, wavelength, and spectroscopy, and two protocols are available in the Supporting Information for laboratory instruction. With the growing ubiquity of 3D printing technology and the inexpensive peripheral parts, the SpecPhone has the potential to widen student accessibility to hands-on training in analytical spectrophotometry.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.5b00654.

Protocol 1: Image Processing and Calibration (PDF, DOCX)

Protocol 2: The Beer–Lambert Law (PDF, DOCX)

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Notes

The authors declare no competing financial interest.

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