S1: 3D Printing the Components of the OPN Spec

Chris Stewart and John Giannini*

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

* Email: giannini@stolaf.edu

We describe here how to 3D print the various components for the OPN Spec. As with other OPN instruments that we have developed,[1, 2] we used the free version of DesignSpark Mechanical (RS Components, Ltd., Corby, Northamptonshire, UK) to design each part of the OPN Spec. This software is available on the following website: http://www.rs-online.com/designspark/electronics/eng/page/mechanical (readers should click on the appropriate 32- or 64-bit icon under the "Download DesignSpark Mechanical" heading near the bottom of the page). We have also provided the CAD and STL files for each component as Supporting Information (S2 and S3, respectively), so that readers can print or modify them to fit their educational or research needs. In addition, we provide the general print dimensions and volumes for these parts in Table S1-1 to assist readers in determining whether their 3D printers can accommodate these components or whether alternative printing arrangements should be made.

Table S1-1. Approximate Print Dimensions (*l* x *w* x *h*) and Volumes (Build, Support) for the Parts of the OPN Spec

Part	Dimensions (cm)	Volumes (cm^3)
Cuvette Holder	3.55 x 4.00 x 4.20	45.60; 4.85
Lid	3.55 x 3.55 x 1.75	20.00; 0.65
Plug	1.80 x 1.80 x 1.60	2.65; 0.20
Tube for Flashlight	3.00 x 3.55 x 7.70	42.65; 6. 85
DVD Rotator Body	6.00 x 5.00 x 3.00	57.90; 4.20
DVD Rotator Post	1.00 x 1.00 x 7.00	5.85; 3.20

With respect to the individual pieces of the OPN Spec, like the OPN Colorimeter,[2] we designed the cuvette holder (S2A, S3A) to house a standard plastic cuvette (external dimensions: 1.25 cm x 1.25 cm x 4.5 cm) and the corresponding lid (S2B, S3B) to fit over the top (Fig. S1-1, top). As with the OPN Colorimeter,[2] the cuvette holder contains

a small tube in the back that holds a plug (S2C, S3C) for the light dependent resistor (LDR), which we use for the detector.

The tube for the light source (S2D, S3D) will hold a Coast G20 LED inspection light, which has a 16-mm diameter (Fig. S1-1, left). However, readers can change the inner diameter of the light tube and its length to hold other small LED flashlights. Alternatively, readers can make tubes that hold larger LED flashlights, and we provide some examples below (Figs. S1-2 and S1-3; Table S1-2).

The holder of the diffraction grating (S2E, S3E) also houses a post (S2F, S3F), which can hold part of a CD or DVD (Fig. S1-1, right). Typically, we use double-sided tape to hold the CD or DVD in place.

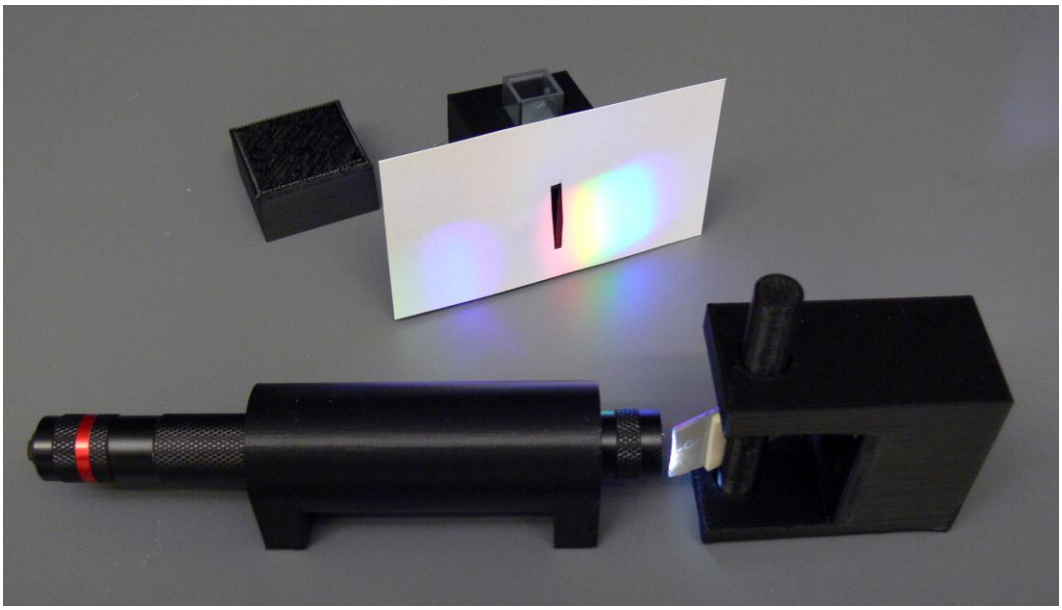

Figure S1-1. 3D-printed version of the OPN Spec, which can hold a Coast G20 flashlight.

STL Files and Test Prints

As with the OPN Scope and OPN Colorimeter,[1, 2] we used the free version of DesignSpark Mechanical to create the STL files for the OPN Spec (Export Options >> 3D Print (*.STL) >> Options), setting the "resolution" to "custom" with a deviation of 0.05 mm and an angle of 0.1 degrees to create smooth curves and distinct corners in the

parts. However, please keep in mind that different 3D printers or 3D printing software may have different error tolerances, so we again suggest that readers print out a few test pieces first (e.g., part of a tube or a portion of the cuvette holder or lid) to make sure that the above settings will generate the desired shapes and dimensions. These test prints should further indicate whether readers need to change any of the dimensions for the components in the CAD files, so that the parts will fit together as desired.

3D Printing

As with the OPN Colorimeter,[2] we printed the components for the OPN Spec on a FlashForge Creator Pro 3D Printer (FlashForge USA, Rowland Heights, CA), using 1.75-mm diameter SainSmart Black ABS Filament as both our build and support material. In addition, we again used MakerBot Desktop as our firmware and Simplify3D as our slicing program.[2] Readers, however, could use other 3D printers, filament, or software instead.

Also, as we have discussed in previous papers,[1, 2] readers who do not have a 3D printer can still upload their STL files to a website like www.makexyz.com or https://www.3dprint-uk.co.uk, which will print and ship parts to you for a fee (usually based on the volume of filament used). For example, in October 2016, we uploaded the STL files for each of the components for the OPN Spec to www.3dprintingpricecheck.com, which estimated that it would cost around $55 (total) to print these six parts using either the MakeXYZ[3] or 3DprintUK[4] websites (not including any taxes or shipping). However, to obtain an exact quote, readers should upload their STL files to their preferred 3D printing website.

Hazards

As explained in our OPN Colorimeter paper,[2] the major hazard that we have found when 3D printing parts for the OPN Spec using the FlashForge Creator Pro is that the

platform for this printer becomes rather hot when in operation (approximately 98° C when printing ABS material). As a result, please be careful when removing a part after a print job has finished, or consider waiting until the platform has cooled slightly before removing the part.

In addition, depending on the particular filament and 3D printing method used, the process of printing a part can release harmful nanoparticles into the air, which can cause a variety of health problems, ranging from asthma attacks to strokes to cardiac or respiratory arrest. As a result, please exercise caution when working around the 3D printer, and make sure to use proper protective equipment (including masks or respirators) when necessary. We further suggest that readers keep their 3D printers in a well-ventilated space to reduce any risks associated with these nanoparticles.

We also include the following link to a PDF from Carnegie Mellon University, which provides a number of additional 3D printing safety tips that are very useful to know.

The Light Source

We have found that many different types of tactical LED flashlights work well in the 3D-printed version of the OPN Spec, and we have had success using the Coast G20, Outlite A100, Outlite WT03, UltraFire, SkyWolfEye, and GeakLight flashlights (Fig. S1-2 from left to right).

Figure S1-2. LED flashlights that we use with the OPN Spec. From left to right, the Coast G20, Outlite A100, Outlite WT03, UltraFire, SkyWolfEye, and GeakLight flashlights.

Of course, some of these flashlights provide brighter and/or wider beams than others, and each model also has its own unique dimensions. As a result, we have designed separated 3D-printed holders for three of these flashlights (the UltraFire, SkyWolfEye, and GeakLight models; S2G01 – S2G03, S3G01 – S3G03; Fig. S1-3A), and we include their general print dimensions and volumes in Table S1-2 below. Given these designs, each flashlight should slid smoothly into its respective tube, and we also place small amounts of this poster tack underneath the handle of these lights to help keep them level and steady on the tabletop (Fig. S1-3A).

Table S1-2. Approximate Print Dimensions (*l* x *w* x *h*) and Volumes (Build, Support) for Different OPN Spec Flashlight Holders

Part	Dimensions (cm)	Volumes (cm^3)
Tube for UltraFire	4.00 x 4.00 x 4.50	31.20; 0.70
Tube for SkyWolfEye	4.00 x 4.00 x 3.50	29.50; 0.70
Tube for GeakLight	4.00 x 4.00 x 3.50	30.70; 0.70

In addition, according to the website www.3dprintingpricecheck.com, it would cost between $7.20 and $13.10 to print out one of these tubes on either the MakeXYZ[3] or 3DprintUK[4] websites (not including any taxes or shipping costs), which would slightly reduce the cost of 3D printing the parts for the OPN Spec (by approximately $3 per instrument) if replacing the Coast G20 flashlight with another model.

For the larger Outlite A100 and WTO3 flashlights, we have found that, given their size, a 3D-printed light tube is not necessary. Instead, we simply lay these flashlights on a tabletop and hold them in place using removable poster tack or mounting putty (Fig. S1-3B). By eliminating the need for a light tube altogether, this approach would reduce the 3D printing costs of the OPN Spec by $10 to $15 if using either the MakeXYZ[3] or 3DprintUK[4] websites.

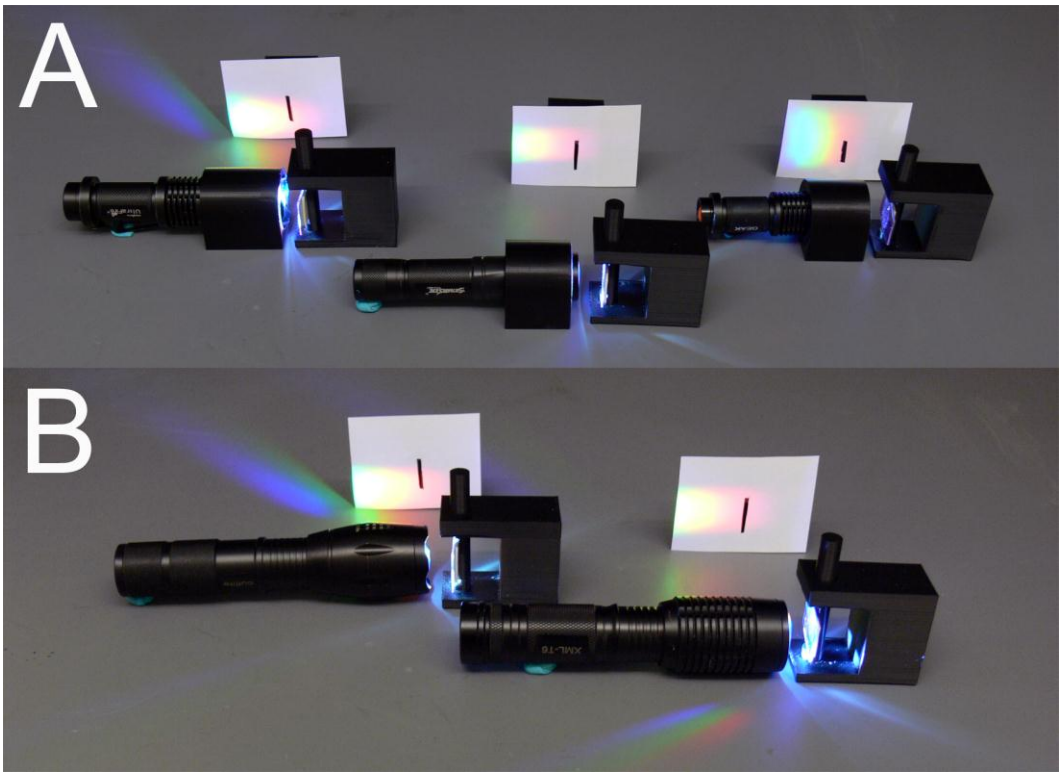

Figure S1-3. Using other LED flashlights with the OPN Spec. (A) Tubes for the UltraFire, SkyWolfEye, and GeakLight flashlights (from left to right). (B) Placing the Outlite A100 and WT03 flashlights in front of the DVD on the tabletop (from left to right) and holding them in place with removable poster tack.

The Light Dependent Resistor (LDR) and Digital Multimeter

As with the OPN Colorimeter,[2] we generally use an inexpensive light dependent resistor (LDR) as our detector in the OPN Spec (Fig. S1-4A, showing a PDV-P8103 photocell by Advanced Photonix, Inc., which has a 500-kOhm "dark" resistance). Typically, we purchase these LDRs from www.digikey.com for approximately 85 cents each. We then place the LDR into the 3D-printed plug (S2C, S3C), which we insert into the back tube of the cuvette holder (S2A, S3A). Then, we connect the leads of the LDR to a digital multimeter to take our readings. Over the past year, we have used several different multimeters in these experiments, and we have bought these devices online or at a regional hardware store for between roughly $5 and $25 each (Fig. S1-4B).

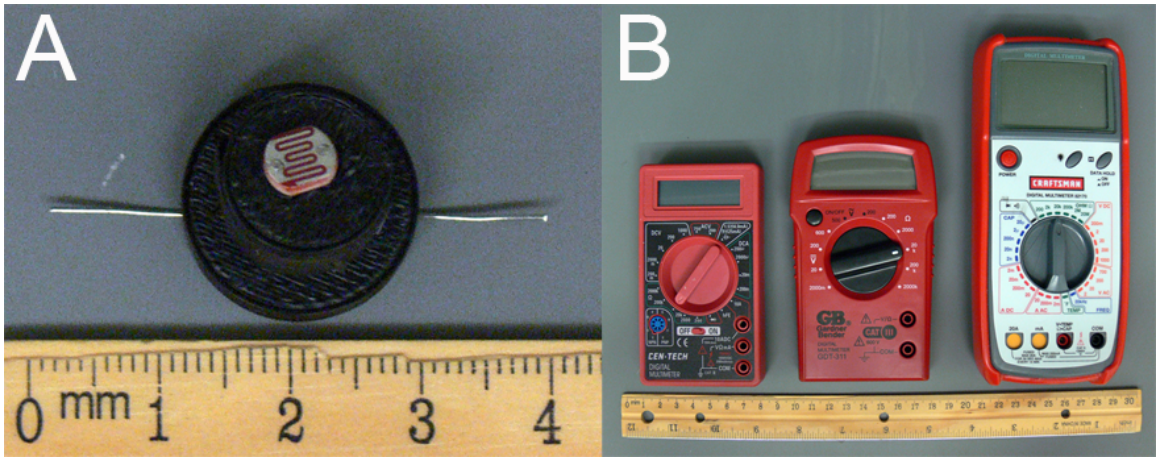

Figure S1-4. The Light Dependent Resistor (LDR) and multimeters that we use with our OPN Specs. (A) PDV-P8103 LDR by Advanced Photonix, Inc. mounted in the back plug for the OPN Spec. (B) Cen-Tech (left), Gardner Bender (center), and Craftsman (right) digital multimeters.

Of course, readers can use other LDRs or multimeters instead, but we suggest conducting a sufficient number of pilot tests with these items in advance to make sure that the OPN Spec is generating useful results. For example, in the assay to generate a simple Absorbance spectrum for 40 µL of Methylene Blue solution (100 mg/L) suspended in 3 mL of deionized water (S5), we typically obtained resistance readings between 5.8 and 293 kOhms for an empty chamber (depending on the amount of time that the batteries had been in use and the placement of the various components).

Similarly, in the other Methylene Blue assay described in the Supporting Information (S6), we generally obtained readings between 3.2 and 44.4 kOhms for an empty chamber (again, depending upon the placement of the OPN Spec components and amount of time that the batteries had been in use). Despite this wide range of values in the two assays, which resulted in useful Absorbance readings in both sets of experiments, readers may still need to recalibrate the set-up if another LDR or flashlight is used or the concentration of another chemical tested.

The CD or DVD that Serves as a Diffraction Grating

To make the diffraction grating that generates our spectra,[5-14] we typically use a standard commercial DVD because we have found that DVDs tended to generate a better spectrum than the CDs that our school had in stock. For the diffraction grating itself, we cut a small wedge out of the DVD (Fig. S1-5) and then trim the top and bottom of that wedge to create a rectangle that is approximately 3.5-cm x 2.5-cm (l x h). We then use double-sided tape to hold the DVD to the flat portion of the rotating post (after placing the post into the holder; Figs. S1-1 and S1-3). Once assembled, the post should turn freely, enabling students to "dial in" different colors (and, thus, different wavelengths) for their experiments.

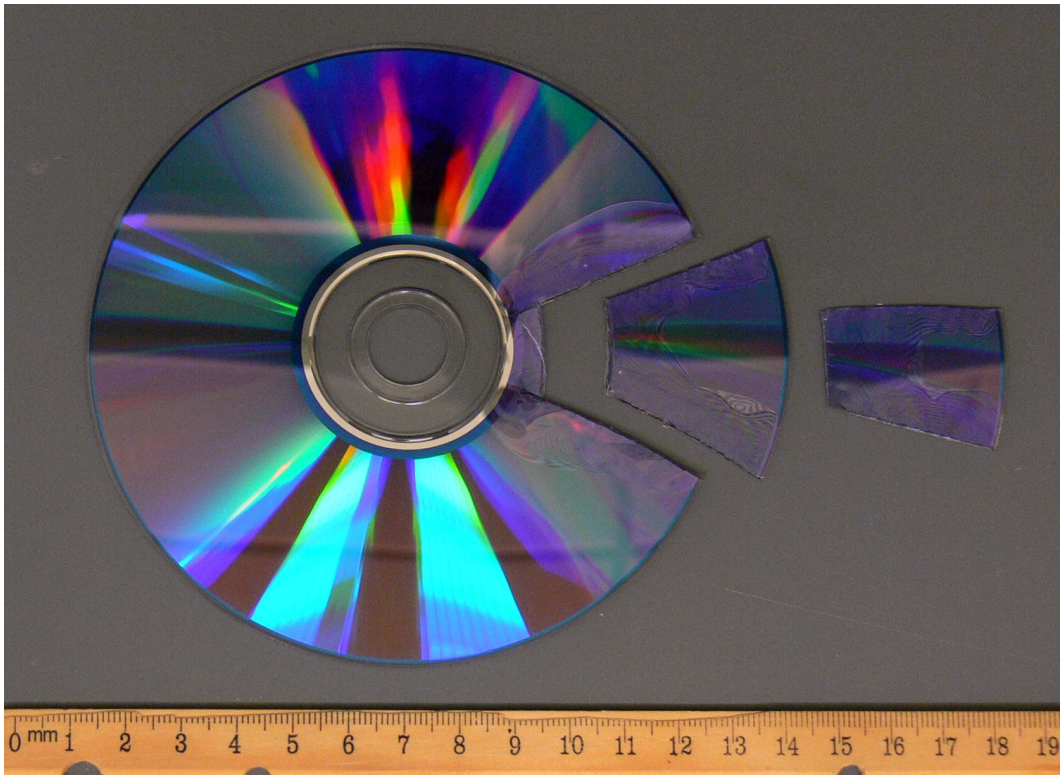

Figure S1-5. Making the diffraction grating from a DVD.

Also, given our results, it appears that this set-up is an improvement upon the OPN Colorimeter – although the OPN Spec still does not appear to be specific for a particular wavelength (e.g., 450 nm exactly). Nevertheless, as with the OPN Colorimeter,[2] teachers and students can still use the OPN Spec to generate standard curves or other graphs for instructional or educational purposes, such as conducting an assay to determine protein concentration or enzyme activity or generating a simple Absorption spectrum for various chemicals in solution.

Using an Index Card to View the Spectrum Generated by the CD or DVD

In addition, on the front of our cuvette holders, we place a small (13-cm x 8-cm) index card or similar piece of white cardstock (e.g., 9-cm x 5-cm) to better view the spectrum generated by the CD or DVD (Figs. S1-1 and S1-6), and we hold these cards in place using small amounts of removable poster tack or mounting putty. Also, before

attaching each card to the face of a cuvette holder, we use a single-edge razor blade to cut a 0.5-cm x 2.0-cm slit into the face (centered around the mid-line), which should be wide enough to allow light to pass through the slit in the cuvette holder. Readers, however, can cut narrower slits, which should allow for a narrower band of wavelengths to pass through the solution and strike the resistor.

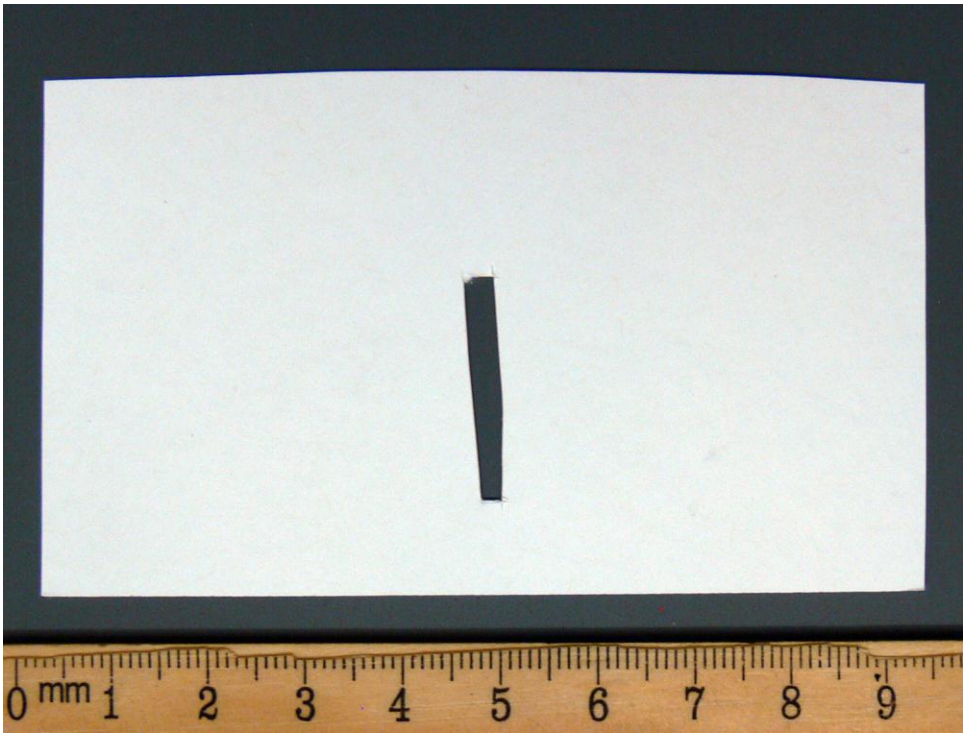

Figure S1-6. Using an index card or a piece of white card stock to view the spectrum generated by the CD or DVD.

Alternatively, readers can print out a cuvette holder that has a narrower slit in its front face, and we have made 2.0-, 1.0-, and 0.5-mm models for this very purpose (S2A01 – S2A03 and S3A01 – S3A03, respectively). Of course, readers can try printing even narrower slits with their own set-up (we stopped at a 0.5-mm wide slit given the apparent precision of our 3D printer).

A Cap and Lid to Hold a Small Test Tube

Finally, as with the OPN Colorimeter,[2] instead of a cuvette, readers can use a small test tube with the OPN Spec (specifically, a VWR Disposable Culture Tube, No. 47729-

572, which is roughly 13 mm in diameter and 100 mm tall). We have included the CAD and STL files for a lid (S2H, S3H) and cover (S2I, S3I) that will fit over such a test tube and hold it in place (Fig. S1-7).

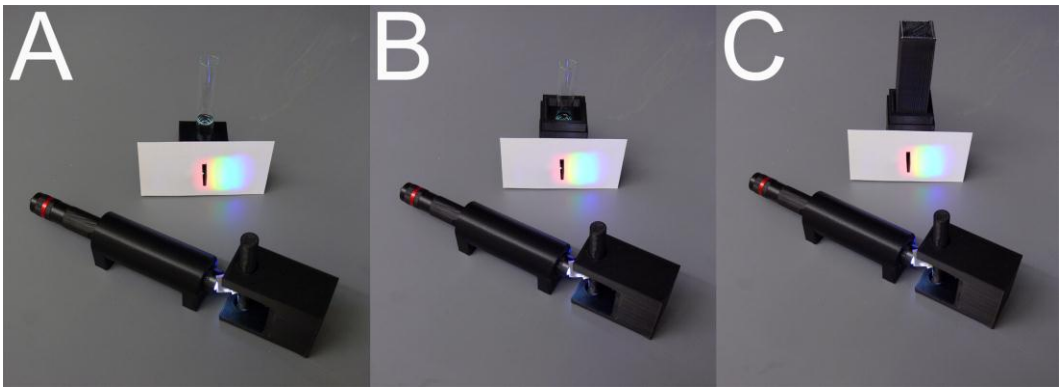

Figure S1-7. A lid and cap to fit a 13-mm x 100-mm test tube in the OPN Spec.

These are the same files contained in the Supporting Information for our OPN Colorimeter paper,[2] and we include their 3D printing dimensions and volumes in Table S1-3 below. Also, for the reasons discussed in that supplement, readers may ultimately find it more economical to simply purchase a box of 100 polystyrene cuvettes (e.g., for $13 to $20 online at October 2016 prices) than to print out multiple copies of those two parts (for roughly $13 per set at October 2016 prices on the MakeXYZ[3] or 3DprintUK[4] websites according to www.3dprintingpricecheck.com) – particularly because these cuvettes can be cleaned and reused after each lab activity.

Table S1-3. Approximate Print Dimensions (*l* x *w* x *h*) and Volumes (Build, Support) for an OPN Spec Lid and Cap that Will Fit a Small Test Tube.

Part	Dimensions (cm)	Volumes (cm^3)
Lid for Test Tube	3.55 x 3.55 x 2.50	22.30; 0.65
Cover for Test Tube	2.35 x 2.35 x 7.05	25.90; 0.30

Helpful Hints for the OPN Spec

Given our experiences in testing and using the 3D-printed version of the OPN Spec, we provide the following helpful hints for its use.

First, to keep each component of the OPN Spec in place on the tabletop during an experiment, we typically use small amounts of removable poster tack or mounting putty. This approach also enables students to move the pieces around before beginning an assay, which may make it easier to adjust the placement or intensity of the light beam striking the cuvette holder. However, once students have started their experiment, they should not move any of the components of the OPN Spec (including the position of the flashlight) since these changes could affect the intensity or color of the light striking the cuvette and LDR, which could render subsequent readings incomparable to earlier ones. Also, instead of using poster tack or mounting putty, readers can try using Velcro strips or Scotch Extreme ® fasteners to fix the various parts of the OPN Spec in place – especially if attaching them to a thin plastic sheet or wooden board, which may make them easier to set up and use (Fig. S1-8). However, in these instances, we suggest that readers first test out the placement of these parts in advance, so that their final positions will lead to useful test results for the students.

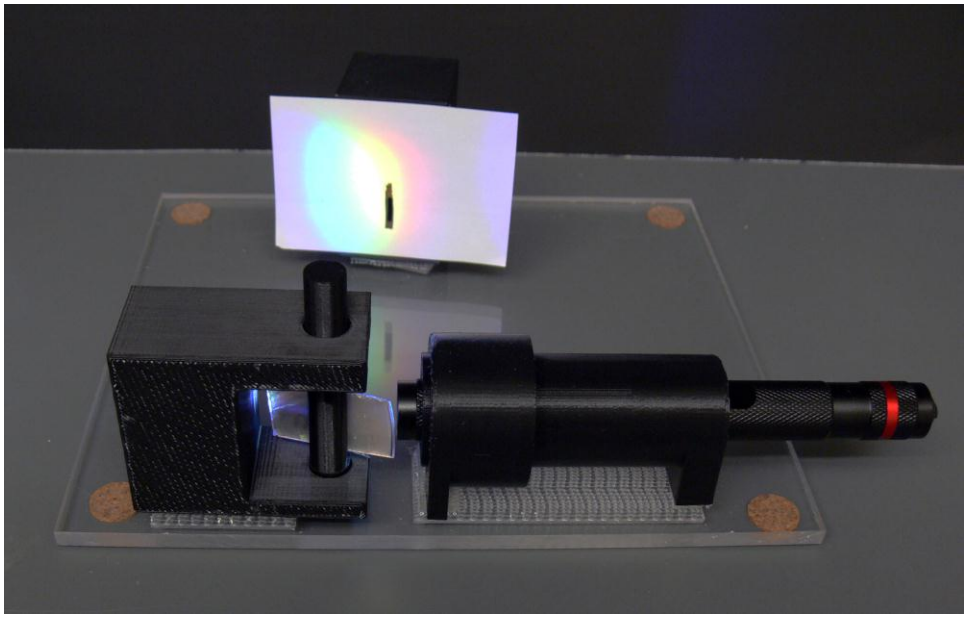

Figure S1-8. Using Scotch Extreme Fasteners ® to attach the components of the OPN Spec to a thin sheet of clear plastic. Note that this set-up also uses a 3D-printed adapter that fits the flashlight tube for the OPN Colorimeter.

Second, unlike the light tubes for the OPN Colorimeter,[2] the light tubes for the OPN Spec have "feet" at each end to hold them upright since the tubes do not fit directly into the face of the cuvette holder. However, for readers who want to use their OPN Colorimeter light tubes with the OPN Spec, we have designed an adapter that slides over one end of an OPN Colorimeter light tube to provide the necessary additional support (S2J, S3J; Fig. S1-8). Readers can also print another adapter that fits into the front face of the cuvette holder for the OPN Colorimeter and contains a slit for allowing a narrow band of light to pass through it (S2K, S3K).

According to www.3dprintingpricecheck.com, it would cost between roughly $11.25 and $13.80 (total) to print these two pieces on either the MakeXYZ[3] or 3DprintUK[4] websites, and we include the printing dimensions and volumes for these pieces in Table S1-4 below. Also, to cut down the cost of these adapters, readers could simply use removable poster tack or mounting putty to hold a white index card with a narrow slit in it (Fig. S1-6) to the face of an OPN Colorimeter cuvette holder, which would eliminate the need for the second adapter (and reduce the above costs by $4.70 to $6.50).

Table S1-4. Approximate Print Dimensions (*l* x *w* x *h*) and Volumes (Build, Support) for Adapters that Will Fit the OPN Colorimeter Light Tube and Cuvette Holder.

Part	Dimensions (cm)	Volumes (cm^3)
Light tube adapter	4.00 x 4.00 x 2.50	16.25; 0.70
Cuvette adapter	3.00 x 3.50 x 2.50	17.40; 0.50

Third, to display a relatively tall and wide spectrum on the front face of the cuvette holder, we place our flashlights up close to the CD or DVD (Figs. S1-1, S1-3, and S1-7). In addition, we tend to position the cuvette holder so that its front face is at least 5 cm away from the center of CD or DVD since this distance allows for a bright light to strike the cuvette (Figs. S1-1, S1-3, and S1-7). Of course, readers can place the cuvette holder further back (e.g., 10 or 15 cm away from the CD or DVD), which should broaden the spectrum that strikes the face of the cuvette holder (making it easier to

"dial in" a narrower band of wavelengths). We also position the cuvette holder so that the light beam falls directly onto the LDR, and we typically slide a small rectangular piece of white paper into the empty chamber (before beginning our experiments) in order to better see where the light falls.

Fourth, to cut down on the amount of ambient light that can strike the cuvette, readers can turn off the overhead lights in their classroom or lab when using the OPN Spec to run an assay. During this time, instructors can use a clear 25-W bulb (or similar faint light source) to light the room. However, depending upon the nature of the lab exercise, this approach may not be feasible, especially for safety reasons.

Otherwise, many of the other "Helpful Hints" for using the OPN Spec are similar to those for the OPN Colorimeter.[2] For convenience, we summarize those suggestions below.

At the outset, readers should turn on their flashlights and multimeters at least 10 minutes before using the OPN Spec to let these devices warm up and stabilize before conducting an experiment. During this time period, readers should further record and graph the output of their resistance values to determine how much warm-up time is actually necessary. Depending upon the results, readers may also want to have their students do the same before beginning their experiments.

Also, unlike the OPN Scope and OPN Colorimeter,[1, 2] the 3D-printed version of the OPN Spec has only one compression fitting: namely, where the plug for the LDR (S2C, S3C) fits into the tube in the back of the cuvette holder (S2A, S3A). While readers can change the dimensions of these two parts in DesignSpark Mechanical to ensure a tighter fit, we simply place a small piece of electrical tape on the cylindrical part of the plug, so that it stays in place when inserted into the tube. Also, even though readers can place small amounts of electrical tape on the post (S2F, S3F) before sliding it into the CD or DVD holder (S2E, S3E), we have found that this step usually is not necessary

since these two pieces fit together rather smoothly on their own. Of course, depending on the exact tolerances of their 3D printers, readers may need to adjust these dimensions in the free version of DesignSpark Mechanical, which is why we recommend printing out a few test pieces first.

As with the OPN Colorimeter,[2] readers may also need to use "jumper wires" with "alligator clips" on them to connect the leads of their LDRs to the probes on their multimeters (Fig. S1-9).

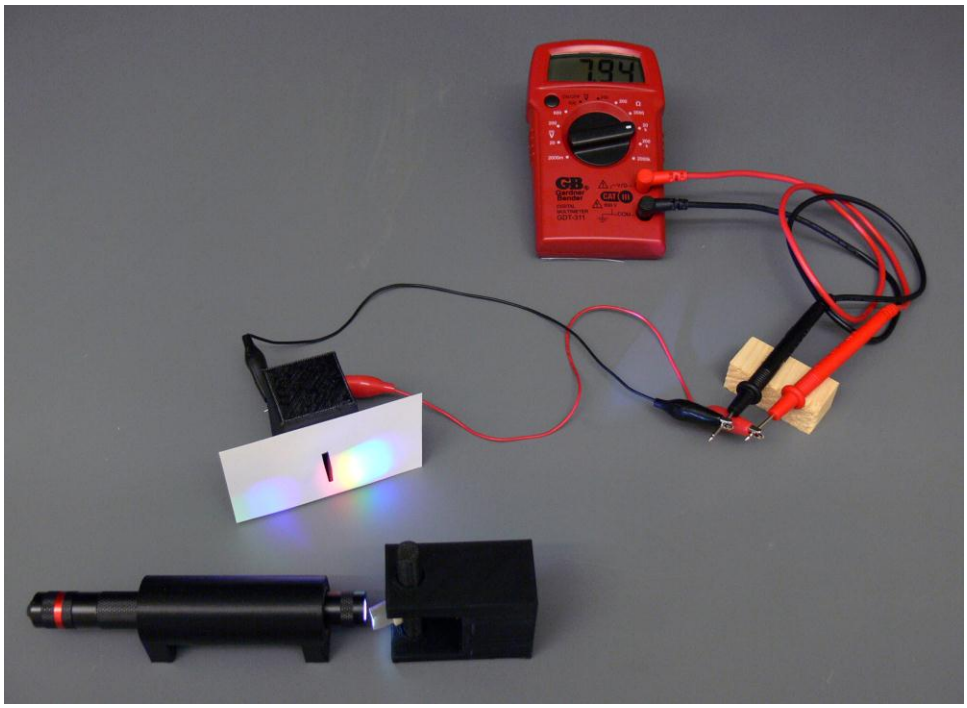

Figure S1-9. Connecting the probes of a digital multimeter to the leads of a light dependent resistor (LDR) using "jumper wires" with "alligator clips" on them.

In addition, besides securing the pieces of the OPN Spec in place before beginning an experiment, instructors may also want to have their students tape their flashlights into their tubes after setting up their OPN Specs. This way, the light sources should not accidentally change position during an assay, which could render subsequent readings incomparable to earlier ones.

Instructors should also make sure that the placement of the various components of the OPN Spec results in an initial reading on the multimeter that will lead to useful results as the experiment progresses. For example, in the various Methylene Blue assays that we conducted (S5, S6), we found that an initial reading for an empty chamber that ranged between 5 and 293 kOhms (S5) or 3 and 44 kOhms (S6) tended to generate useful as data as the experiment progressed.

Nevertheless, we still recommend that instructors conduct some pilot tests of their planned lab activity in advance to gain a better understanding of the resistance values that students should obtain during the exercise. As explained in the Supporting Information for our OPN Colorimeter paper,[2] such test runs would be especially helpful if using a different LDR or LED flashlight than the ones described above (or a different chemical assay than the one described in Supporting Information S5 and S6).

Finally, because the resistance of the PDV-P8103 photocell is inversely proportional to the intensity of the light striking it, the proper formula for measuring Absorbance (A) with this set-up is $A = \log_{10}(T/I)$ without a negative sign in the equation.[2] In this formula, T denotes the intensity of the transmitted light passing through the cuvette and I denotes the intensity of the incident light striking the cuvette (as measured by the resistance value for an empty chamber).

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