

S4: Building a Wooden or PVC Version of the OPN Colorimeter

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We describe here how to build an alternative version of the OPN Colorimeter, using parts available at most hardware stores (e.g., wood or PVC board, PVC tubing, electrical tape, etc.) or online (e.g., a commercial light meter). As with the OPN Scope, which we described in an earlier report,¹ we include a list of the basic materials needed to build this instrument (along with their estimated cost at April 2016 prices) in Table S4-1. Readers, however, should note that many of these items (especially the wood or PVC board and the PVC pipe) will make more than one OPN Colorimeter. We further provide the dimensions for these wooden and PVC pieces in lumber units (i.e., inches) since those are the standard units of measurement for these types of materials and their related tools.

Table S4-1. Description and Approximate Cost of the Parts for a Wooden or PVC Version of the OPN Colorimeter

Part	Approx. Cost
¾-inch thick wood or PVC board	\$2 to \$20
¾-inch PVC pipe (Schedule 40; 5 ft. long)	\$2
Commercial light meter	\$25 to \$35
LED flashlight	\$2 to \$15
Metal hinges	\$1 to \$3
Ten wood screws (#6; 1¼ in. long) or glue, epoxy, etc.	\$2 to \$5
One manila folder or similar item	\$1
Colored cellophane or similar material (for the filter)	\$1 to \$5
Electrical tape	\$1 to \$3
AA or AAA batteries (one four-pack)	\$2
Total	\$39 to \$92

In addition, while readers can build this version of the OPN Colorimeter using hand tools, it can be assembled more quickly and easily using power tools (e.g., a chop or table saw, a portable drill or drill press, a disk or belt sander, etc.), which should be available at most high schools, colleges, or universities. If unfamiliar with such tools, however, please review the Hazards section below given the significance of the associated dangers.

The Light Meter and Light Source

Since the light meter and light source are the most critical (and also the most costly) components of the OPN Colorimeter, we discuss them first.

We purchased our light meters (a Pyle PLMT12 and a Dr. Meter LX1330B) on Amazon for roughly \$25 and \$35 (Fig. S4-1A). We chose these light meters because they both have a 200-lumens setting, which can report results up to four digits. Of course, readers can use other commercial light meters instead. Alternatively, readers can use a light dependent resistor (LDR) connected to a digital multimeter for their detectors, as described in the Supporting Information for the 3D-printed version of the OPN Colorimeter (S1). However, because an LDR is much smaller than the hand-held sensor from a light meter, readers may want to adjust the dimensions of the OPN Colorimeter accordingly. Towards this end, we provide some alternate designs for more compact versions of the OPN Colorimeter, which will house an LDR, near the conclusion of this supplement.



Figure S4-1. The commercial light meters and LED flashlights that we use with our OPN Colorimeters. (A) Pyle PLMT12 (left) and Dr. Meter LX1330B (right) light meters. (B) Coast G20 LED inspection light (top) and 35-lumen LED keychain light (bottom).

As our light source, we typically use a Coast G20 LED inspection light, which we bought for roughly \$11 at a regional hardware chain (Fig. S4-1B, top). Alternatively, readers can use other flashlights, and we have found that a 35-lumens keychain LED light with an outer diameter of roughly $\frac{3}{4}$ inches is sufficiently bright to generate useful results (Fig. S4-1B, bottom). This particular light will further will fit snugly into a $\frac{3}{4}$ -inch PVC tube without the need for any additional tape or other modifications. In our experience, such keychain lights are also relatively inexpensive (e.g., we purchased the light shown in the bottom of Figure S4-1B for roughly \$2 at the same regional hardware chain where we bought the Coast G20 light).

The Outer Shell for the OPN Colorimeter

To build the outer shell for this version of the OPN Colorimeter, we used $\frac{3}{4}$ -inch thick board that we purchased from the “spare wood” bin at a regional hardware chain for roughly \$2 total. In general, the outer shell consists of seven parts: a 6-inch x 6-inch top and bottom; two 3-inch x 6-inch sides, and three 4½-inch x 3-inch walls (Fig. S4-2A). We further drilled a 1½-inch diameter hole roughly 1 inch above and 1⅛ inch to the left of the lower right corner of the front wall to hold the PVC tube for the LED flashlight (Fig. S4-2A, bottom middle) and a ⅛-inch diameter hole the same distance from lower right corner of the inside wall to provide a path for the beam from the flashlight, so that it can reach the hand-held sensor (Fig. S4-2A, center). We also cut a small notch near the upper left corner of the left wall, so that the lid would close tightly over the cord for the hand-held light sensor (Fig. S4-2B, upper left corner; see also Fig. S4-4, lower right corner). Of course, because other light meters may have larger or smaller hand-held light sensors, readers may need to adjust the dimensions of these pieces (including the locations of the holes for the PVC tube and light path) in order to fit their particular model.

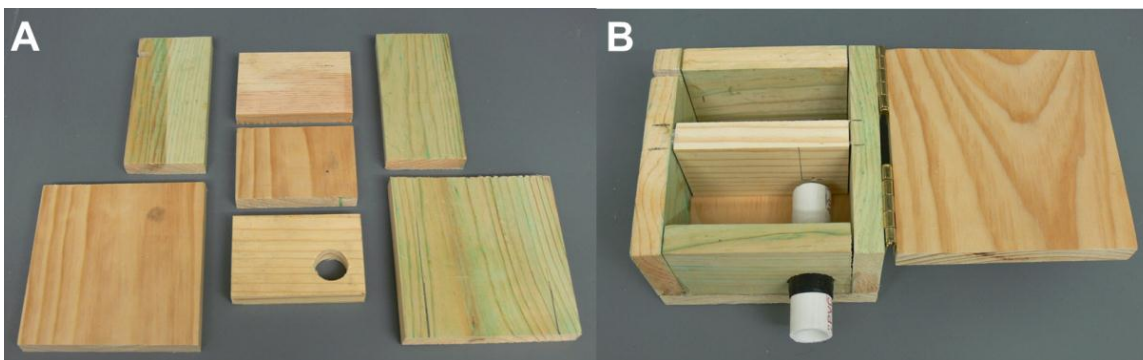


Figure S4-2. The unassembled (A) and assembled (B) pieces of the outer shell for a wooden version of the OPN Colorimeter, which can hold a Pyle PLMT12 light meter.

Also, while we used hot glue to hold together the pieces of the outer shell shown in Figure S4-2 above, readers can instead use super glue, epoxy, or wood screws to accomplish this task. In addition, we used two metal hinges to attach the lid and included a wooden knob on top for easy opening. Also, we placed four felt pads near the bottom corners of the base, so that the instrument would sit flat on a table or benchtop.

Of course, readers can instead make this outer shell using PVC board, which might be preferable due to its rigid nature (e.g., given the wood that we used, we found that the lid on one of our OPN Colorimeters had warped so significantly over the course of several months that it needed to be replaced). However, because commercial PVC boards tend to be white and fairly reflective, we recommend coating the inner sides with a few layers flat black spray paint before assembling the outer shell. That way, light should not reflect off of the inner sides of the PVC board, which might cause spurious readings by the light meter. While readers can also coat the inner sides of a wooden OPN Colorimeter with flat black spray paint, we have found that this step is not necessary to obtain useful readings given the light meters that we use.

The Cuvette Holder

We generally make our cuvette holders by cutting apart a manila file folder, and we provide below a design for a simple holder that will fit a standard cuvette (external dimensions: 1.25 cm x 1.25 cm x 4.5 cm). Specifically, we cut out a rectangular piece manila that is roughly 2¾ inches x 1¼ inches (70 mm x 35 mm) to fit around a standard cuvette and a smaller rectangular piece that is roughly 1¼ inches x ½ inches (5 mm x 10 mm)

to serve as the “bottom” of the holder (Fig. S4-3A). We make the holder by placing a standard cuvette on the larger manila piece, and we then mark where to fold or cut the piece using dashed or dotted lines, respectively (Fig. S4-3A). Next, we use an X-Acto knife or similar tool to cut out a “window” in the holder, and we then fold the pieces around the cuvette, using tape to hold everything together (Fig. S4-3B). Of course readers could use other materials (or other designs) for their cuvette holders, and we have had success with cardboard, poster board, and tag board.

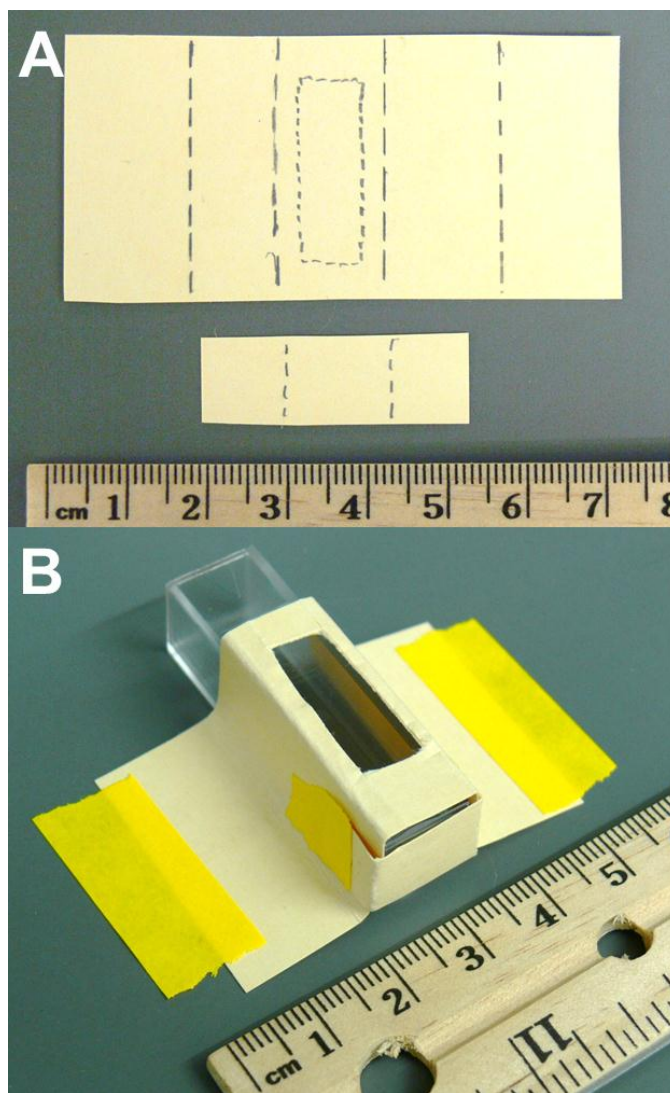


Figure S4-3 The cuvette holder for the OPN Colorimeter. (A) Readers should fold along the dashed lines and cut along the dotted lines to assemble the holder. (B) The assembled holder (held together with tape).

Once folded, the holder can then be placed up against the front wall of the back chamber in the OPN Colorimeter and held there using tape (Fig. S4-4). In the process, please make sure to align the “window” in the cuvette holder with the small hole in the wall, so that the light beam will pass through the cuvette and strike the light sensor (Fig. S4-4).



Figure S4-4 Placing the cuvette holder in the back chamber of the OPN Colorimeter. Note that we used a piece of cardboard as our holder in this design.

The Cellophane Filter

For our filters, we use rectangular pieces of colored cellophane that are roughly 2½ inches x 2 inches (60 mm x 55 mm).² We then tape the cellophane to a 3½-inch x 2½-inch (90-mm x 65-mm) piece cut from a manila file folder, which contains a 1½-inch x 1½ -inch (45-mm x 45-mm) “window” in it (Fig. S4-5). Once assembled, these filters can simply be placed up against the back wall in the front chamber of the OPN Colorimeter (see Figure S4-7 for an example). To keep the filter upright, readers can further fold the far end of the manila holder slightly to create a simple stand. Also, instead of using a manila file folder, readers can again use cardboard, poster board, tag board, or any other similar material for their filter holders.

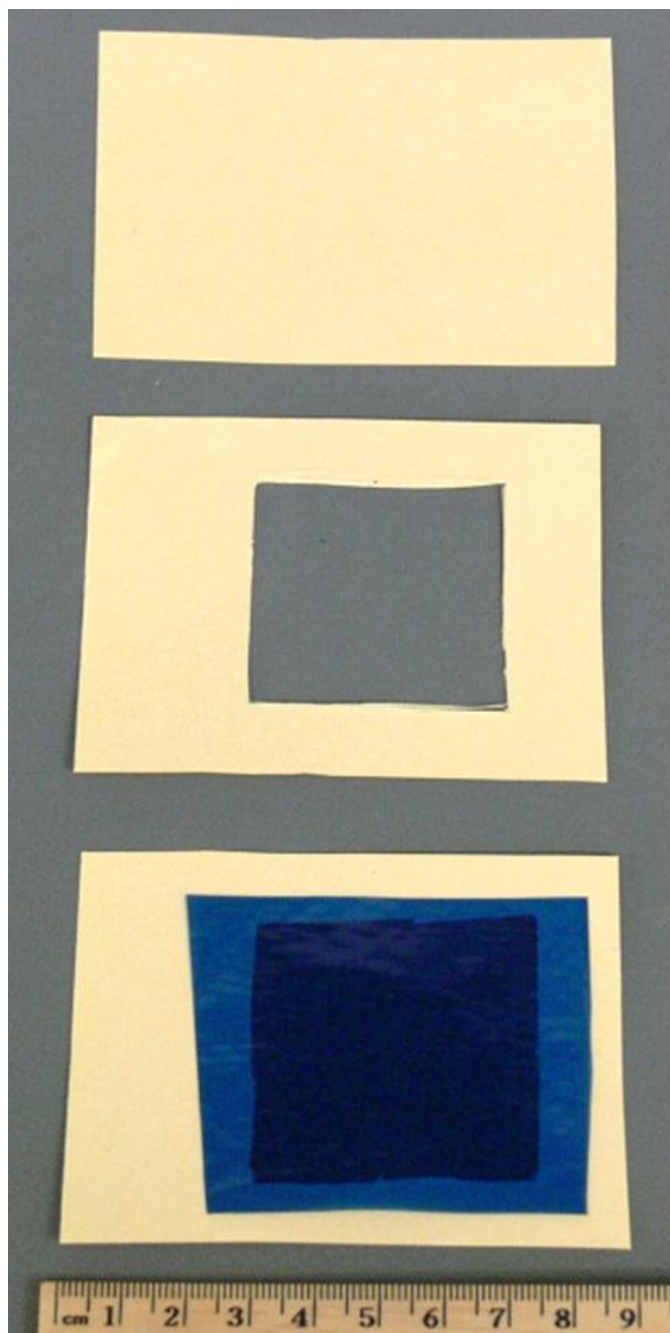


Figure S4-5. Creating a holder for the filter using part of a manila file folder and a piece of colored cellophane.

Although these filters are not specific for a particular wavelength (e.g., 450 ± 5 nm exactly), we have found that they can still be used to generate useful data for analytical or instructional purposes (e.g., creating a standard curve to determine protein concentration or a graph that shows the various rates of different enzyme-catalyzed reactions). We have further included in the Supporting Information for the 3D-printed version of the OPN Colorimeter (S1; Fig. S1-5;

Table S1-2) the transmission spectra for two popular brands of colored cellophane (Edukit and Hygloss), which we purchased on Amazon for between \$6 and \$8 each.

Alternatively, readers could use other types of filters to fit their needs, such as colored photographic “gels” for digital cameras that are available on Amazon for around \$10 to \$20 per collection or more sophisticated optical filters that can be purchased on eBay or other suitable marketplaces for used lab equipment. If so doing, however, please make sure to test the filters in advance to make sure that they will generate useful results during the lab activity.

The Tube for the Flashlight

For the tube that holds the flashlight, we use a 3½-inch long piece of ¾-inch Schedule 40 PVC pipe, which has an outer diameter of roughly 1.05 inches. As with the PVC version of the OPN Scope,¹ we use a compression fitting to secure this tube in place, wrapping it with a few layers of electrical tape, so that it would fit tightly into the hole in the front face of the outer shell (Fig. S4-2B).

Also, because the Coast G20 light has a smaller outer diameter than the inside of the PVC tube, we wrap this flashlight with several layers of electrical tape in two different places, so that it fits more smoothly into the PVC pipe (Fig. S4-6). Of course, if using the LED keychain light described above, this step would not be necessary.



Figure S4-6. Wrapping a Coast G20 flashlight with electrical tape in two places, so that it will fit smoothly into a ¾-inch PVC tube.

Assembling the OPN Colorimeter

Once the outer shell of the OPN Colorimeter has been built, putting together the rest of the instrument is relatively straightforward. In particular, readers need only place the PVC tube (wrapped in a few layers of electrical tape) through the hole in the front face and then place the hand-held light sensor into the back compartment, making sure that it is aligned with the beam of light passing through the hole in the middle wall (Fig. S4-7). We further recommend placing a small “ball” of tape on the back of the sensor to hold it in place and prevent it from moving during the course of the experiment.

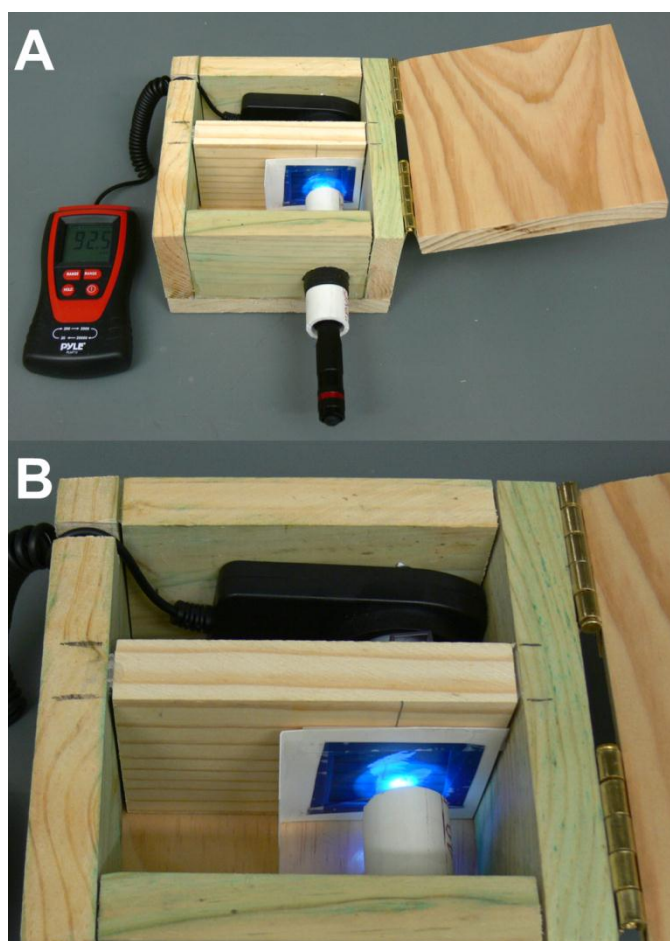


Figure S4-7. An assembled OPN Colorimeter.

Using a Light Dependent Resistor (LDR) for a Detector

As discussed above, readers can alternatively use an LDR and digital multimeter as their detector (instead of a commercial light meter). If so, readers could simply drill two holes for the leads of the LDR into the back wall of the OPN Colorimeter, making sure to align those holes on

opposite sides of where the beam of light will strike the wall. However, if readers instead want to make a smaller version of the OPN Colorimeter, which is closer to the dimensions of the 3D-printed model, we provide some alternate designs here.

Specifically, to build the outer shell for the first alternative version of the OPN Colorimeter, we used three 2-inch x 2½-inch pieces of wood that were ¾ inches thick and one 2-inch x 3-inch piece of plywood that was ½ inch thick – roughly the width of a standard cuvette (Fig. S4-8). To identify the center of the three 2-inch x 2½-inch pieces, we drew diagonal lines between the opposite corners on each piece (Fig. S4-8, top row). We next drilled a 1⅝-inch diameter hole through the center of the first 2-inch x 2½-inch piece to hold the PVC tube for the flashlight (Fig. S4-8, top left), a ⅛-inch diameter hole through the center of the second 2-inch x 2½-inch piece to provide a pathway for the light beam (Fig. S4-8, top middle), and a ⅝-inch diameter hole through the center of the third 2-inch x 2-inch piece to hold a #4 cork stopper (⅝-inch diameter) that will contain the LDR (Fig. S4-8, top right).

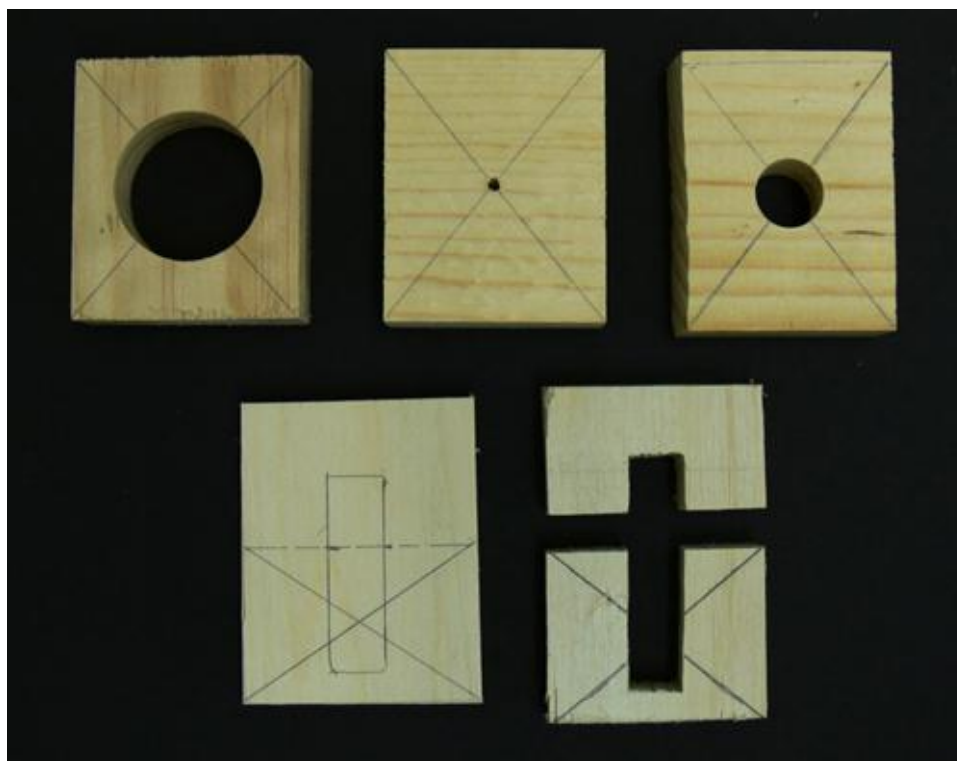


Figure S4-8. The unassembled pieces for a wooden version of the OPN Colorimeter, which can hold a light dependent resistor (LDR) placed in a cork stopper.

For the cuvette holder, we first drew a dashed line across the middle of the 2-inch x 3-inch piece of plywood to divide it into two 2-inch x 1½-inch pieces (Fig. S4-8, bottom left). This line, however, could be drawn at other levels, such as the 2-inch mark. We then identified the center of the “bottom” half of the piece by drawing two diagonal lines between the opposite corners as described above. We next placed a standard cuvette onto that piece roughly ½ inch from the bottom edge and traced its outline, so that we could later cut that portion away from the plywood to create a chamber for the cuvette. Then, we used a band saw to cut the piece of plywood in half (along the dashed line that we had previously drawn) and to cut out each piece for the cuvette holder, creating two roughly symmetric pieces – i.e., a bottom and a top or “lid” (Fig. S4-8, bottom right).

We next assembled these pieces to create this alternate version of the OPN Colorimeter, using fast-drying super glue to hold the parts together. Specifically, we glued the front piece (with the 1⅛-inch hole in it) to the piece with the ⅛-inch hole in it, making sure to align the two holes so that they were centered (we used the X’s on these two pieces as a guide). Then, we glued the bottom half of the cuvette holder to the back of the second piece, again making sure to center the space for the cuvette. Next, we glued the final piece (with the ⅝-inch hole in it for the cork stopper) to the other side of the cuvette holder, centering that hole over the open space for the cuvette.

Importantly, when gluing these pieces together, we typically let one sit on top of another for several minutes before moving on to the next piece. We took this approach because we discovered that pressing the pieces together tightly or placing them in a vice (a standard carpentry practice) tends to compress the wood to such an extent that the cuvette will no longer fit inside its chamber.

At times, however, we needed to use wood putty to fill in some gaps between the wooden pieces in a finished OPN Colorimeter, but this did not cause any issues down the line. Also, if using a vice to hold the pieces of wood together when gluing them, please consider including some thin, but strong spacing material (e.g., hard plastic or other similar material) on at least

one side of the ½-inch plywood in order to provide enough clearance for the cuvette after all the pieces have dried.

Once assembled, these four wooden pieces will form the body for this version of the OPN Colorimeter, and a standard plastic cuvette should slide smoothly into the chamber (Fig. S4-9). Also, if necessary, consider using a disk or belt sander to smooth out the sides, making them flat and even.



Figure S4-9. The assembled pieces for a wooden version of the OPN Colorimeter, which can hold a light dependent resistor (LDR), without the lid in place.

To make a filter for this version of the OPN Colorimeter, we simply cut a 1-inch diameter circle from a piece of colored cellophane and trimmed it as necessary to fit into the hole in the front face for the PVC tube. We then wrapped a piece of ¾-inch PVC tubing that was roughly 3½ inches long with a few layers of electrical tape and slid it into to the front hole to hold the colored filter in place (Figs. S4-9 and S4-10). Finally, to prevent this tube from “drooping” over time (especially with the Coast G20 light), we typically place a small (¾-inch tall) wooden block underneath the tube as a support (Fig. S4-10).

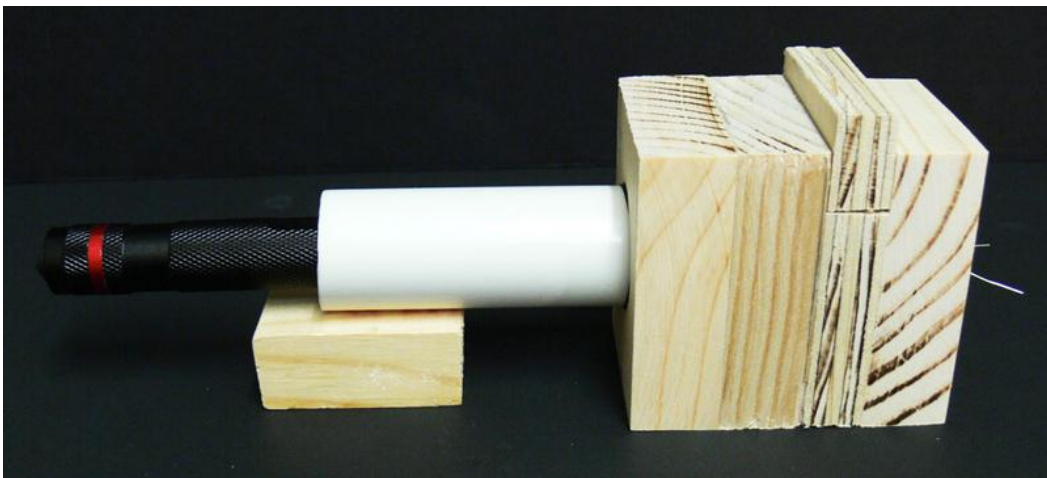


Figure S4-10. A fully-assembled, alternate version of the OPN Colorimeter, which can hold a light dependent resistor (LDR), with the lid in place and a small block of wood under the PVC tube to prevent it from “drooping” over time.

To hold the LDR in place, we typically use a #4 cork stopper with a $\frac{5}{8}$ -inch diameter that we purchased at a regional hardware chain for roughly 25 cents.³ We also cut roughly $\frac{3}{8}$ inches off of the bottom (i.e., smaller) end of this stopper to provide enough room for the LDR (otherwise, the LDR will stick out into the empty chamber for the cuvette). In addition, we drill two small holes through the cork stopper (using a $\frac{1}{16}$ -inch bit) for the leads of the LDR (Fig. S4-11, left). After fitting those leads through the holes in the cork, we place the stopper into the hole in the back of the OPN Colorimeter. Alternatively, readers could drill a smaller (e.g., $\frac{1}{2}$ -inch diameter) hole into the back piece, which should prevent the cork stopper from passing all the way into the hole and, thus, provide enough room for the LDR without needing to cut down the cork (Fig. S4-11, middle). We have also had success using a #6 cork stopper (with a $\frac{3}{4}$ -inch diameter at its widest span) pushed partially into a $\frac{5}{8}$ -inch diameter hole (Fig. S4-11, right).

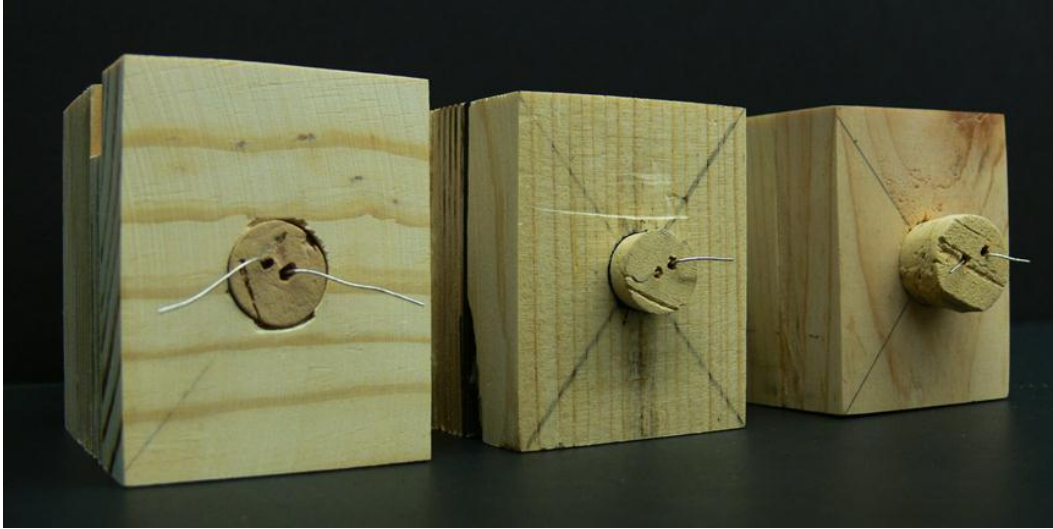


Figure S4-11. Using a cork stopper to hold the LDR in the back of the OPN Colorimeter. From left to right, a #4 stopper in a $\frac{5}{8}$ -inch diameter hole, a #4 stopper in a $\frac{1}{2}$ -inch diameter hole, and a #6 stopper in a $\frac{5}{8}$ -inch diameter hole.

Once assembled, this version of the OPN Colorimeter can be connected to a digital multimeter and used in the same fashion as the 3D-printed version (S1), and we include the protocols that we used to test this and other wooden versions of the OPN Colorimeter (along with some representative data) in Appendix S4-A.

Alternatively, readers can make a wooden version of the OPN Colorimeter that uses a $\frac{3}{4}$ -inch Schedule 80 (gray) PVC pipe cap to cover the cuvette. To do this, first, follow the same steps set forth above to make the three 2-inch x $2\frac{1}{2}$ -inch pieces of $\frac{3}{4}$ -inch thick wood for the previous version (Fig. S4-12, top row). Then, use a 2-inch x $2\frac{1}{2}$ -inch piece of plywood for the cuvette holder (note the slightly smaller height of this piece). Next, identify the center of this piece by drawing two diagonal lines between the opposite corners, and center a standard plastic cuvette on the piece roughly $\frac{1}{2}$ inch from the bottom. Then, trace the outline of the cuvette onto the piece of plywood, making sure to extend the lines all of way up to the top edge since the cuvette will not extend that far (Fig. S4-12, bottom row). Finally, cut out the space for the cuvette using a band saw and glue the four pieces together as described above.

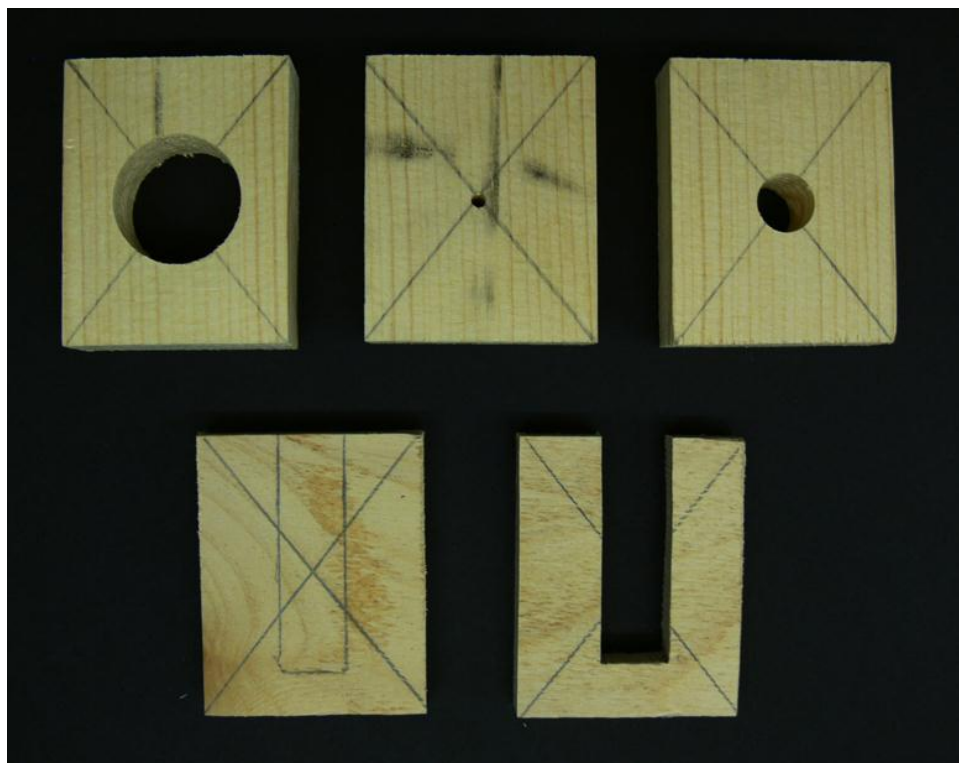


Figure S4-12. The pieces for an alternate version of the OPN Colorimeter, which uses a $\frac{3}{4}$ -inch Schedule 80 PVC cap for the lid to cover the cuvette.

Once assembled, drill a $1\frac{3}{8}$ -inch diameter hole in the top of the OPN Colorimeter centered over the chamber for the cuvette (Fig. S4-13A). Then, place a $\frac{3}{4}$ -inch Schedule 80 PVC pipe cap (wrapped in a few layers of electrical tape) into the hole to cover the cuvette (Fig. S4-13B). As with the previous wooden version of the OPN Colorimeter, readers can then cut out a small (1-inch diameter) piece of colored cellophane for the filter, and they may also need to use a small wooden block to prop up the PVC tube if using a Coast G20 inspection light in this model (Fig. S4-13C). Readers may further note the dark black plastic spacer (i.e., the solid black line) between the $\frac{1}{2}$ -inch thick plywood and the rear wooden piece in this version of the OPN Colorimeter (Fig. S4-13). We needed to include this spacer here because the piece of plywood that we used for this version of the OPN Colorimeter turned out to be slightly thinner than one-half of an inch. As a result, we had to provide a little more room for the cuvette, so that it would fit into the chamber before we glued on the last (i.e., rear) piece to form the OPN Colorimeter body.

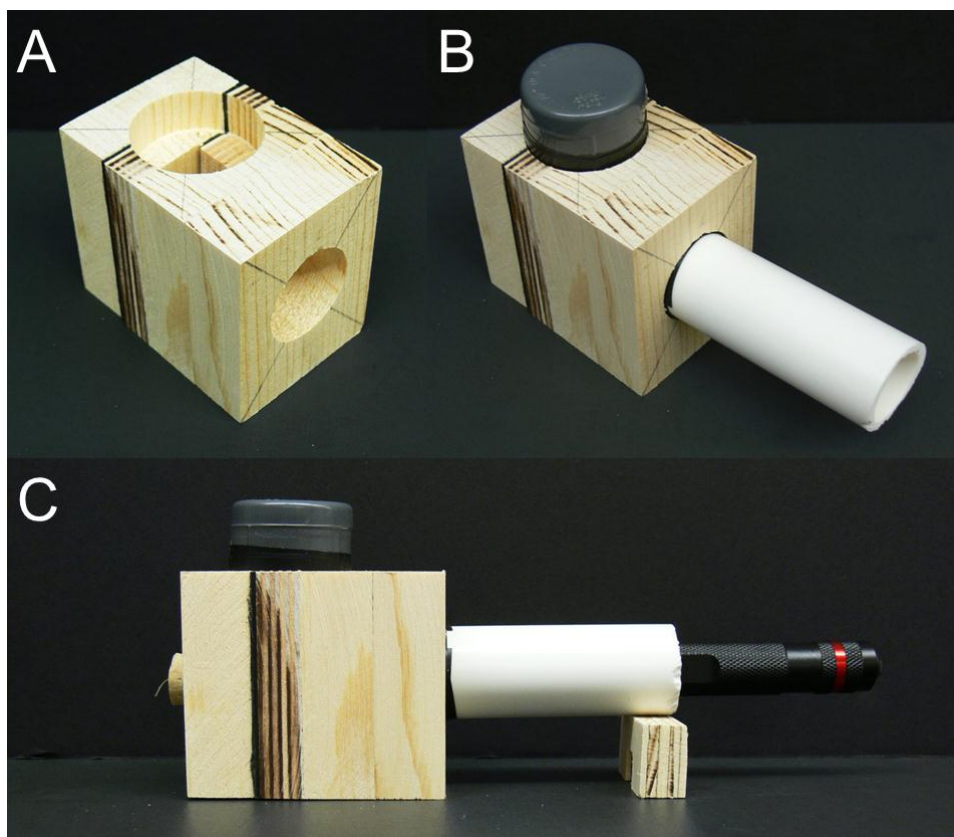


Figure S4-13. A wooden version of the OPN Colorimeter that uses a $\frac{3}{4}$ -inch Schedule 80 PVC cap as a lid to cover a plastic cuvette.

Of course, readers who do not have access to a band saw could easily adapt these designs to make a version of the OPN Colorimeter that holds a small test tube, such as the model described in the Supporting Information for the 3D-printed version of the instrument (S1). To do so, simply replace the $\frac{1}{2}$ -inch-thick piece of plywood with another 2-inch x $2\frac{1}{2}$ -inch piece of wood that is $\frac{3}{4}$ of an inch thick – without making any other cuts to this new piece (Fig. S4-14). Then, glue the four pieces together as described above. Again, if necessary, use a disk or belt sander to smooth out the sides and make them all flat and even.

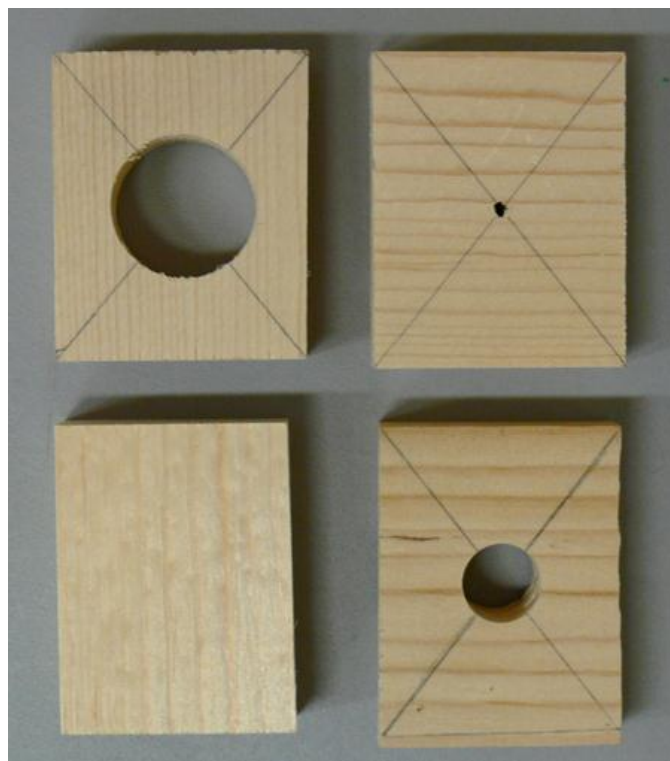


Figure S4-14. The four 2-inch x 2½-inch wooden pieces for an alternate version of the OPN Colorimeter that will hold a small test tube.

Next, drill a small (e.g., 1/8-inch diameter) guide hole roughly 1 inch into the top of the third piece (i.e., the one that will hold the cuvette), making sure to center this hole before drilling (e.g., by drawing two diagonal lines between the opposite corners on the top face). Then, using this guide hole, drill a 1 1/8-inch diameter hole roughly 1/2-inch deep into the top of the piece. Once finished, this hole will overlap slightly with the second and fourth pieces, which should be expected (Fig. S4-15A). The hole itself will serve as a “collar” to hold a 3/4-inch PVC tube in place, which will serve as a cover for the test tube.

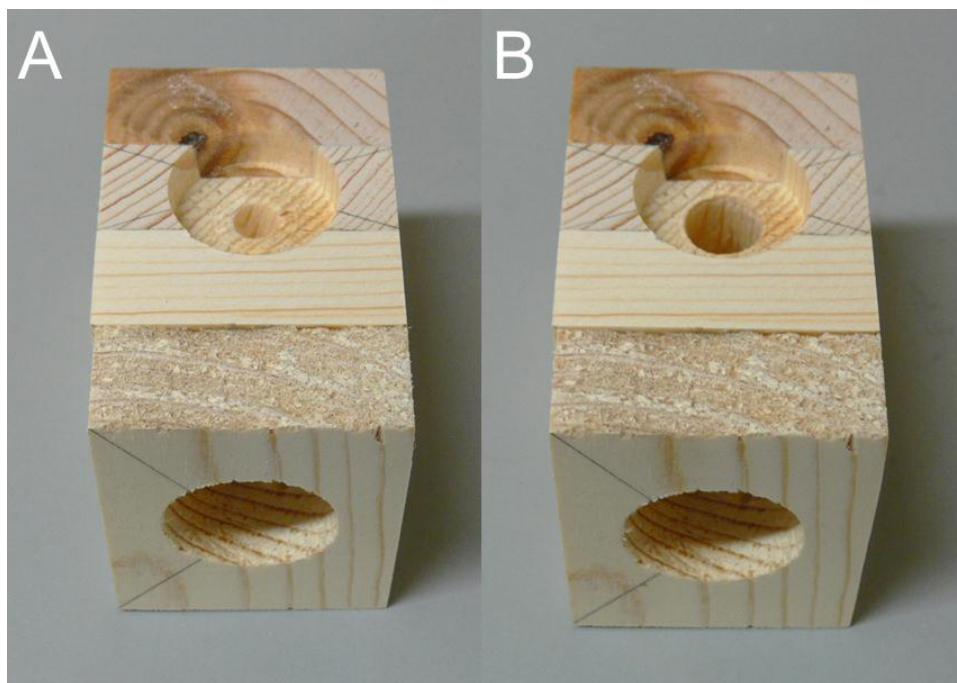


Figure S4-15. Drilling (A) a $\frac{1}{8}$ -inch diameter hole to serve as a collar for a length of PVC pipe that will cover the test tube and (B) a $\frac{1}{2}$ -inch diameter hole to hold the test tube. Note how we drew an “X” on top of the third piece to center the initial hole.

Then, drill a $\frac{1}{2}$ -inch diameter hole deeper into the third piece (roughly $1\frac{1}{2}$ inches), using the same guide hole to center the drill bit (Fig. S4-15B). This hole should end roughly $\frac{1}{2}$ inch from the bottom of the piece and will serve as the holder for the test tube, which has a 13-mm (approximately $\frac{1}{2}$ -inch) diameter. However, in the event this hole is too narrow for the test tube, readers can use sandpaper, a round or half-round wood file, or a Dremmel or similar rotary tool to widen the hole slightly. Of course, for larger test tubes, a larger hole would be necessary.

Also, because this $\frac{1}{2}$ -inch diameter hole for the test tube is smaller than the $\frac{3}{4}$ -inch thick piece of wood, it will be necessary to drill a hole through the third piece, so that the beam from the flashlight can strike the LDR once it is put in place. To do so, simply drill through the hole in the back piece, making sure to use the same $\frac{5}{8}$ -inch (or $\frac{1}{2}$ -inch) drill bit that was originally used to create that hole. Alternatively, readers can create a path for the beam of light by drilling through the $\frac{1}{8}$ -inch hole in the second wooden piece. If doing so, please make sure that the $\frac{1}{8}$ -inch drill bit used here is long enough to pass all the way through the piece that holds the test tube.

Once finished, a test tube can be placed into this version of the OPN Colorimeter (Fig. S4-16A) and covered with a 2- to 3-inch length of $\frac{3}{4}$ -inch Schedule 40 PVC tubing that has a corresponding pipe cap on it to block out the light (Fig. S4-16B). We have also found that it helps to place a “rolled up” piece of black construction paper inside the PVC tube to further keep out the light. Alternatively, readers could cover the test tube with a length of Schedule 80 PVC pipe, which is thicker than Schedule 40 PVC tubing and gray in color and, thus, should block out the light much better (Fig. S4-16C).

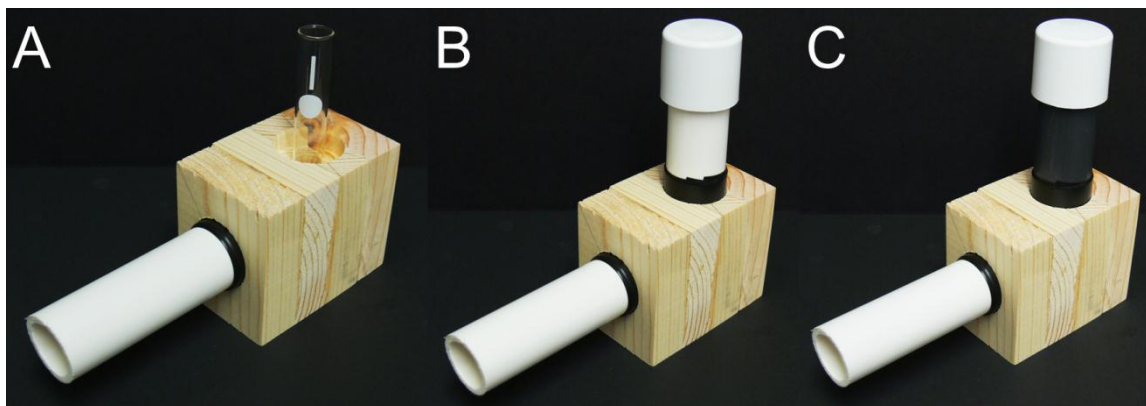


Figure S4-16. An assembled version of the OPN Colorimeter that can hold a small test tube. (A) Placing the test tube in the OPN Colorimeter. (B) Using a $\frac{3}{4}$ -inch Schedule 40 PVC tube and corresponding pipe cap to cover the test tube. (C) Using a $\frac{3}{4}$ -inch Schedule 80 PVC tube and corresponding pipe cap to cover the test tube.

Finally, readers can instead use part of a “4 x 4” post (which actually measures $3\frac{1}{2}$ inches x $3\frac{1}{2}$ inches) as the body a similar version of the OPN Colorimeter. To do so, simply cut a $3\frac{1}{2}$ -inch long piece from such a post (although other lengths will also work). Then, as described above, identify the center of the front, back, and top sides of the wooden block by drawing diagonal lines between the opposite corners of each face (Fig. S4-17A). Next, drill $\frac{1}{8}$ -inch guide holes roughly 1 inch into the center of each one of these three faces. Then, as described above, drill a $1\frac{1}{8}$ -inch diameter hole roughly $\frac{1}{2}$ -inch deep into the center of the top face. This hole will hold the PVC tube and cap that will cover the test tube (Fig. S4-17B). Next, using the same guide hole, drill a $\frac{1}{2}$ -inch diameter hole roughly 2 inches deeper into the block (i.e., ending approximately 1 inch from the bottom). This hole will hold the test tube (Fig. S4-17A).

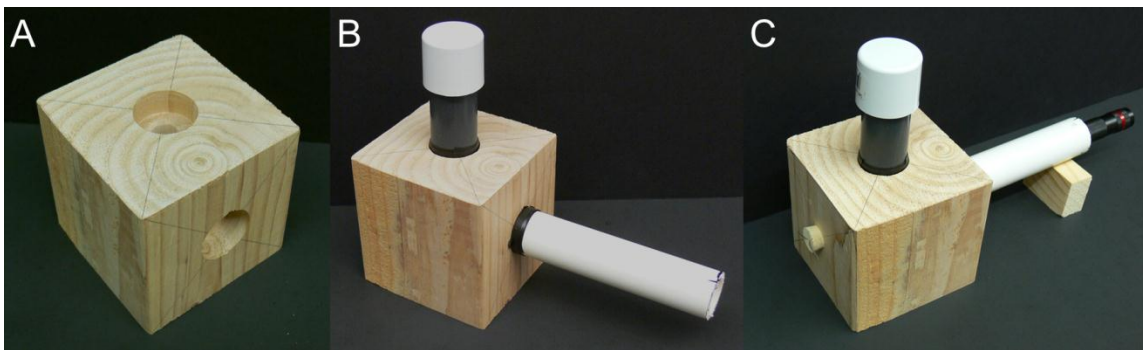


Figure S4-17. Making a wooden version of the OPN Colorimeter out of a “4 x 4” post. (A) The wooden block with holes drilled in the front, top, and rear faces. (B and C) An assembled OPN Colorimeter with PVC tubes in the front and top to hold the light source and cover the test tube, respectively.

Then, using the guide hole in the center of the front face of the wooden block, drill a $1\frac{1}{8}$ -inch diameter hole roughly $\frac{1}{2}$ -inch deep into that face (Fig. S4-17A). This hole will hold the PVC tube for the light source (Fig. S4-17B). Next, extend the guide hole into $\frac{1}{2}$ -inch diameter hole for the test tube, using a $\frac{1}{8}$ -inch diameter drill bit. This hole will provide a path for the light to strike the solution in the test tube. Finally, drill a $\frac{1}{2}$ - or $\frac{5}{8}$ -inch diameter hole into the center of the back face of the wooden block, roughly $1\frac{3}{4}$ to 2 inches deep (which should extend into the hole for the test tube). This final hole will hold a cork stopper containing the LDR (Fig. S4-17C). Also, to check the alignment of these holes, simply hold the wooden block up to the light and look through the rear hole. You should see light coming through the smaller $\frac{1}{8}$ -inch diameter hole, which should further appear in (or at least very close to) the center of the rear hole.

Finally, we used wood (instead of PVC board) to make these versions of the OPN Colorimeter because, in our experience, it is much easier to obtain a $\frac{1}{2}$ -inch-thick piece of plywood for the cuvette holder than it is to cut down a $\frac{3}{4}$ -inch thick piece of PVC board, so it matches the half-inch thickness of a standard cuvette. Plus, given the small sizes of the wooden pieces, they should not warp substantially over time once they are glued together.

Of course, readers could use PVC board instead of wood to make models of the OPN Colorimeter that would hold a test tube or even a cuvette. For example, similar to the design shown in Figures S4-14 through S4-16, readers could use four pieces of 2-inch x $2\frac{1}{2}$ -inch PVC board that is $\frac{3}{4}$ -inch thick to make a version of the OPN Colorimeter that holds a test tube.

Alternatively, similar to the “4 x 4” version of the OPN Colorimeter shown in Figure S4-17 above, readers could use six small pieces of PVC board to make a “box” that holds a test tube. However, in these instances, readers may need to coat the inner sides of the PVC board with a few layers of flat black spray paint to prevent light from reflecting off of the white PVC. Although we did not find this approach necessary in the wooden models of the OPN Colorimeter that we built (see Appendix S4-A), the brighter surface of the PVC may prove different.

Hazards

As we explained in the Supporting Information for the OPN Scope,¹ hand and power tools are extremely dangerous and the risks of injury associated with them are as significant as they are obvious. Consequently, readers should exercise a tremendous amount care when using any tools to build or assemble this instrument, including wearing the proper eye and ear protection. Also, if unfamiliar with these types of tools, we again recommend that readers work with an experienced craftsperson for safety reasons. Alternatively, as with the OPN Scope, in some schools, building these colorimeters could be assigned as a project for a shop class, which could make them for a science class.

Helpful Hints

Given our experiences with this version of the OPN Colorimeter, we include some helpful hints for its use.

Most importantly, if using a commercial light meter as a detector, we have found that it is frequently necessary to turn on the light meter and flashlight at least 10 minutes before using the OPN Colorimeter to let these devices warm up and stabilize before conducting an experiment. To do this, simply put the light meter and flashlight into the OPN Colorimeter, turn them both on, and close the lid. We have found that such a warm-up period is necessary to avoid any significant drift in the readings, which are likely to decline over time. We further suggest that, when first setting up their OPN Colorimeters, readers record and graph the output of their light meter(s) over this warm-up period to determine how much time is actually necessary. Alternatively, readers can use an LDR instead of a light meter as their detector, but

we still suggest using the same warm-up period to see how the intensity of the LED flashlight changes over time. Using an LDR instead of a commercial light meter may also lower the overall cost of the OPN Colorimeter (depending upon the price of the corresponding multimeter used).

Also, to obtain a useful set of readings, we have found students should position their flashlights in the PVC tube, so that the light meter initially reads around 100 lux for an empty chamber. In our experience, a value of around 100 lux should allow for any subsequent increases or decreases in the readings during the course of the Bradford and lysozyme assays described in the Supporting Information (S5 and S6).

We, nevertheless, recommend that instructors still conduct a test run of the lab activity in advance to have a general idea of the values that students should obtain during the exercise (and, thus, whether their initial readings may be too high or too low for useful results). A test run would be particularly appropriate if using different light meters or flashlights for the experiment or using a different protein or enzyme for the activity. Alternatively, if using an LDR instead of a light meter, we typically use a PDV-P8103 photocell by Advanced Photonix, Inc. (S1), and we usually position a Coast G20 inspection light in the tube, so that the multimeter initially reads between 150 and 350 Ohms for an empty chamber. For more information on using an LDR, including the need to use the Absorbance formula $A = \log_{10}(T/I)$ with the LDR described above, please see the Supporting Information for the 3D-printed version of the OPN Colorimeter (S1).

Relatedly, once students begin taking readings with their OPN Colorimeters, they should not move the flashlight in the PVC tube or the colored filter inside the box since such adjustments will likely render subsequent readings incomparable to earlier ones. As a result, instructors may want to have their students tape their flashlights and filters into place once they have positioned them in the OPN Colorimeter. Students may also want to use removable mounting putty, poster tack, double-sided tape, or similar materials to hold their wooden OPN Colorimeters in place on a benchtop or lab table.

In addition, if using an LDR and multimeter as a detector, readers should note that some multimeters come with probes that cannot clip directly onto the leads of the LDR (Fig. S4-18). In these instances, readers can simply use “jumper wires” with “alligator clips” on them to connect the leads of the LDR to those of the probe.

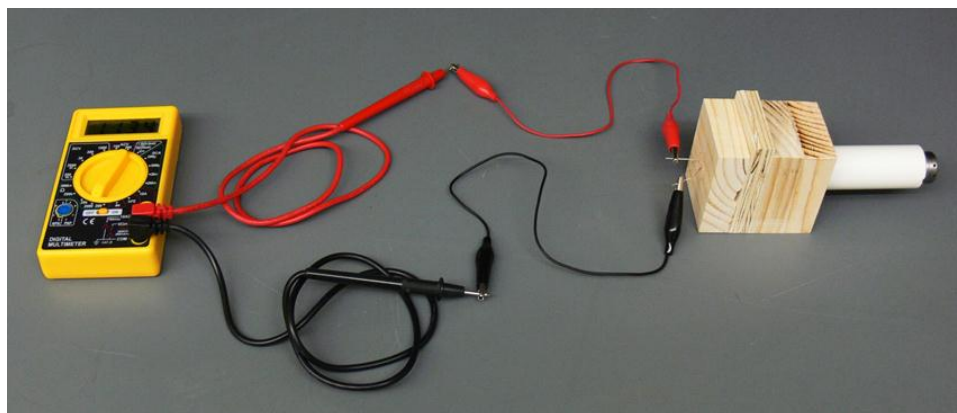


Figure S4-18. Connecting the probes of an inexpensive digital multimeter to the leads of a light dependent resistor (LDR) using jumper wires with alligator clips.

Finally, if making the alternate version of the wooden OPN Colorimeter that holds an LDR, we offer the following woodworking tips. First, even if some of the initial cuts to make the four wooden pieces are a little off (resulting in pieces with slightly different dimensions), these irregularities can be removed either before or after the pieces are glued together by using a disk or belt sander, which should create smooth and flat sides (compare Fig. S4-8 with Figs. S4-9 and S4-10, Fig. S4-12 with Fig. S4-13, and Fig. S4-14 with Figs. S4-15 and S4-16). Second, when drilling holes through these wooden pieces, readers should consider placing a piece of scrap wood underneath the one that they are drilling to avoid chipping or cracking the back of that piece once the bit passes through it. For example, we typically placed two pieces of wood with the same dimensions in a vice that we could center on our drill press and then drilled through the top piece and into the bottom one, which avoided cracking the first piece. Third, while readers can use standard “twist” bits to drill these holes, “paddle” bits or “Forstner” bits may work better since the two specifically designed to drill holes into wood. Relatedly, even though Forstner bits tend to be more expensive than the others, these bits are less likely to inadvertently pierce through the side of a wooden piece since they are designed to drill flat-bottomed holes into wood (paddle bits, on the other hand, tend to have long, pointed tips

whose lengths must be taken into account in advance to avoid inadvertently drilling through a particular piece).

References

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Appendix S4-A: Methylene Blue Assay

To examine the differences between the various wooden versions of the OPN Colorimeter described in this supplement, we used the same Methylene Blue protocol described in Appendix S1-A for the 3D-printed model (S1). Thus, we briefly describe that process here.

We set up each wooden version of the OPN Colorimeter, using a Coast G20 inspection light, a PDV-P8103 photocell, a Craftsman digital multimeter (No. 82170), and a piece of red cellophane as our filter (since the peak absorbance wavelength for Methylene Blue – our test chemical – falls within the red spectrum at 668 nm). We then turned on the light and the multimeter, placed the appropriate lid on the OPN Colorimeter to cover an empty chamber, and recorded the corresponding resistance value, which served as the intensity of the light striking the cuvette or test tube (*I*).

Then, we placed a standard plastic cuvette or small Pyrex test tube (a VWR Disposable Culture Tube, No. 47729-572) into the OPN Colorimeter, added 3 mL of deionized water (DI H₂O), and took a reading, which served as our “zero” value.

Next, we added 5 μ L of a Methylene Blue solution (100 mg/L) to the cuvette or test tube, gently suspending the mixture (5 times) to ensure a thorough mixing. Once finished, we replaced or closed the lid and took another reading.

We then repeated this process, sequentially adding another 5, 10, 20, 40, 80, and finally 160 μ L of the Methylene Blue solution to the cuvette or test tube, recording the corresponding resistance value before moving onto the next volume.

Also, to test the effects of darkening the internal chamber for the cuvette or test tube (including the holes for the light path and cork stopper), we stained these areas with Varathane premium, fast-dying ebony wood stain (Rust-Oleum Corp., Vernon Hills, IL) in two models of the OPN Colorimeter: (i) the version that holds a plastic cuvette and has a Schedule 80 PVC cap as a lid (Fig. S4-13) and (ii) the version made from a “4 x 4” post that holds a small test tube (Fig. S4-17). We then re-ran the Methylene Blue assay on the following day (after the stain had dried), using the same equipment, materials, and procedures.

Finally, as a control, throughout these tests, we ran the same assay with a standard plastic cuvette placed into a commercial spectrophotometer (a Hitachi U-1100) that was set to a wavelength of 668 nm (the peak absorbance wavelength for Methylene Blue in water).^{A1-A3} We further repeated the experiment in triplicate, entered our data into a Microsoft Excel spreadsheet, averaged the results for each instrument tested, and then graphed the final set of data as scatter plots (Figs. S4-A1 and S4-A2).

In particular, for the OPN Colorimeters using an LDR, we used the formula $A = \log_{10}(T / I)$, omitting the negative sign from the Beer-Lambert equation, since the resistance of the PDV-P8103 photocell is inversely proportional to the intensity of the light striking it. 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 associated with an empty chamber). Since the Hitachi U-1100 automatically calculated the Absorbance of each solution, we simply recorded those values in our spreadsheet. Finally, to account for the Absorbance of the DI H₂O and its container, we subtracted the Absorbance value associated with 3 mL of DI H₂O in the cuvette or test tube (i.e., the “zero” value) from each of the subsequent Absorbance calculations.

We found that the wooden versions of the OPN Colorimeter slightly outperformed their 3D-printed counterparts, but were less sensitive than the Hitachi U-1100 commercial spectrophotometer, which we used as a control (Fig. S4-A1). For example, regardless of whether a standard plastic cuvette or small Pyrex test tube was placed in the instrument, the Absorbance readings for the different wooden versions of the OPN Colorimeter were roughly 25% to 75% lower than those of the commercial spectrophotometer over the range of Methylene Blue concentrations that we tested (again, this difference in the readings was likely due to the wider spectrum of light transmitted by the cellophane filters). Nevertheless, each wooden model of the OPN Colorimeter generated a saturation curve that followed the same general trend as the one generated by the commercial device (Figs. S4-A1A, S4-A1C, S4-A1E), including in the linear range (Figs. S4-A1B, S4-A1D, S4-A1F).

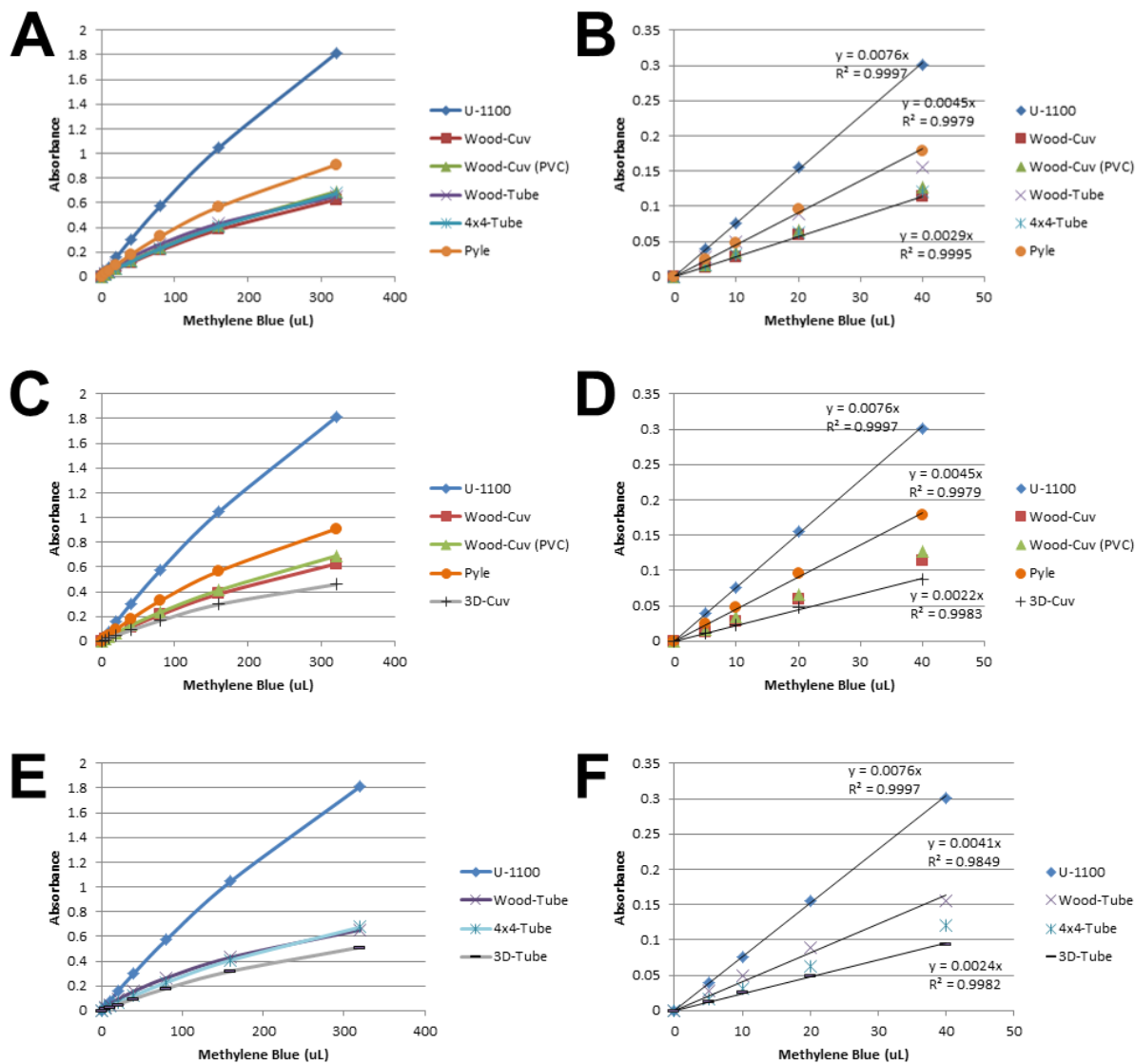


Figure S4-A1. Results of our Methylene Blue assay to test various wooden versions of the OPN Colorimeter, using a plastic cuvette (cuv) or a small Pyrex test tube (tube). (A, C, and E) Scatterplots showing the Absorbance of 0 to 320 μL of Methylene Blue solution (100 mg/L) added to 3 mL of deionized water (DI H_2O) in a cuvette or test tube compared to the results from a commercial (Hitachi U-1100) spectrophotometer and a 3D-printed version of the OPN Colorimeter. (B, D, and F) Scatterplots showing the linear range of this data with accompanying best-fit lines for some of these instruments.

Also, although we were not able to include the equations for all of the best-fit lines in Figure S4-A1 due to space limitations, we have listed this information in Table S4-A1 along with the ratio of the slope of each of these lines to the one generated by the commercial spectrophotometer ($y = 0.00076x$).

Table S4-A1. Equations of the Best-Fit Lines for the Wooden Versions of the OPN Colorimeter in the Linear Range of Our Methylene Blue Tests and the Ratios of Each Slope to the Hitachi U-1100 Data ($y = 0.00076x$).

OPN Colorimeter	Linear Equation (R^2)	Hitachi U-1100 Comparison
Wood-Cuv	$y = 0.00029x$ ($R^2 = 0.9995$)	0.38
Wood-Cuv (PVC)	$y = 0.00032x$ ($R^2 = 0.9989$)	0.42
Pyle	$y = 0.00045x$ ($R^2 = 0.9979$)	0.59
Wood-Tube	$y = 0.00041x$ ($R^2 = 0.9849$)	0.54
4x4-Tube	$y = 0.00031x$ ($R^2 = 0.9991$)	0.41

In addition, we found that staining the inner chamber of the two different wooden versions of the OPN Colorimeter (i.e., the one with a PVC cap for a lid and the one made from a “4 x 4” post) did not dramatically change the Absorbance readings of these instruments (Fig. S4-A2). Specifically, whether or not their inner chambers were stained, each version of the OPN Colorimeter generated similar saturation curves in these Methylene Blue tests (Figs. S4-A2A and S4-A2C), including in the linear range (Figs. S4-A2B and S4-A2D). We believe that these close similarities are likely due to the way in which Absorbance readings are calculated (i.e., by effectively controlling for the initial brightness or darkness of the chamber when measuring the intensity of the incident light I).

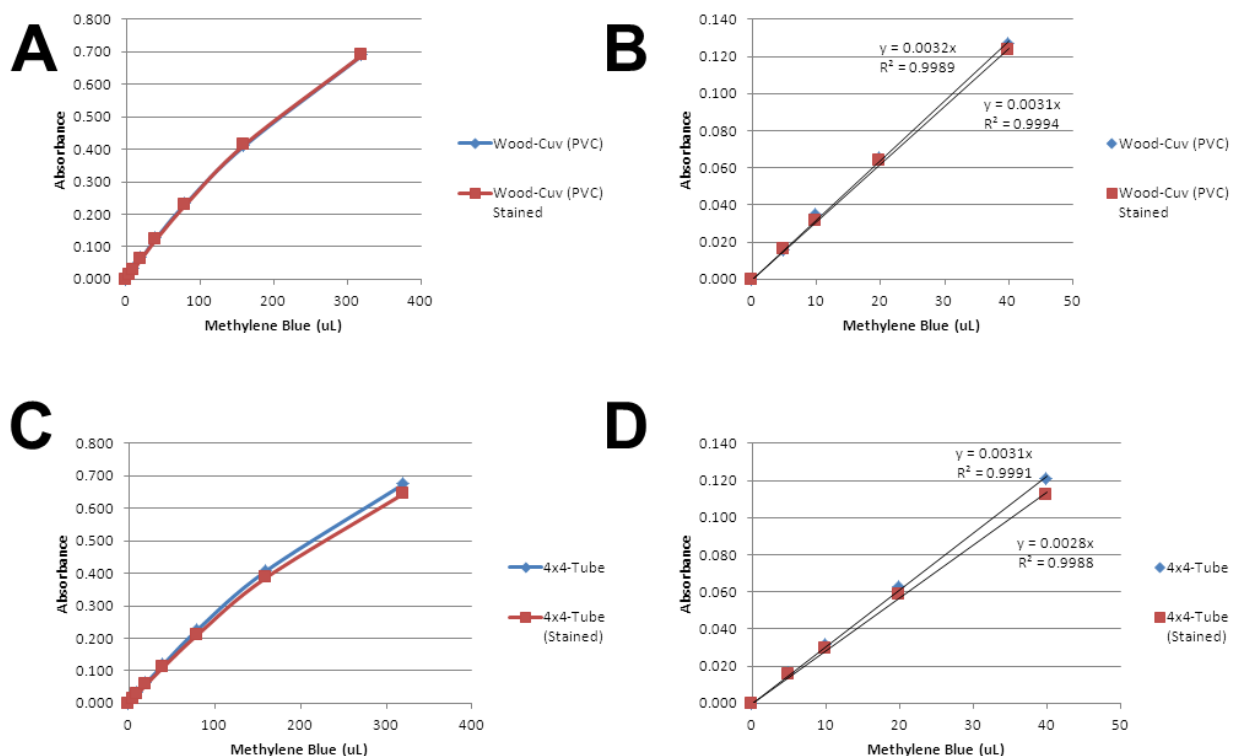


Figure S4-A2. Results of our Methylene Blue assay to test the effects of staining versus not staining the internal chamber of a wooden OPN Colorimeter. (A and C) Scatterplots showing the Absorbance of 0 to 320 μL of Methylene Blue solution (100 mg/L) added to 3 mL of deionized water (DI H_2O) in a cuvette or test tube placed into a wooden OPN Colorimeter with a PVC lid for a cap or one made from a 4x4 post. (B and D) Scatterplots showing the linear range of this data with accompanying best-fit lines for each wooden model of the OPN Colorimeter.

Hazards

As explained in the Supporting Information for the 3D-printed version of the OPN Colorimeter (S1), Methylene Blue is a hazardous chemical that has numerous harmful effects. For example, it can cause serious skin, eye, and respiratory irritation, and it can prove harmful if swallowed or inhaled. The chemical has also caused mutagenic effects and adverse reproductive effects in laboratory animals, and exposure to Methylene Blue in large doses or over prolonged periods of time can cause organ damage. The compound can also ignite if exposed to an open flame, sparks, a hot surface, or other sources of heat or ignition.

As a result, teachers and students should exercise a great deal of caution when working with this chemical, which includes wearing the proper protective equipment (e.g., gloves, goggles, lab coats or heavy clothing with long sleeves, masks or respirators, etc.), avoiding the formation of any vapors or mists when handling the substance, and working in a well ventilated room

away from any possible sources of ignition. In addition, if using Methylene Blue as part of a laboratory exercise, we recommend that instructors prepare diluted solutions of the chemical themselves given the risks described above. In the process, instructors should wear an appropriate dust mask or respirator to protect against inhaling any Methylene Blue particles. Teachers and students should also observe proper lab etiquette when handling the chemical, which includes not eating or drinking around the substance and thoroughly washing their hands after working with the material. Finally, readers should carefully review the Material Safety Data Sheet for this compound before working with it, and they should dispose of any solutions containing Methylene Blue as chemical waste at the conclusion of any lab activity or classroom demonstration (i.e., these mixtures should not be poured down any drains or simply emptied into the trash).

References

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