S4: Building a Wooden Version of the OPN Fluorometer

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In this supplement, we describe how to build a wooden version of the OPN Fluorometer, using items available at most hardware stores (e.g., wood, PVC tubing and couplings, electrical tape, etc.) or online (e.g., a 4x objective lens for a compound microscope, a tactical LED flashlight, a sensitive light dependent resistor or LDR, a digital multimeter, etc.). As with other OPN instruments that we have created,1-3 we include a list of the basic materials needed to build this fluorometer (along with their estimated cost at November 2016 prices) in Table S4-1. Of course, several of these items (particularly the pieces of wood and PVC pipe) will make the parts for many OPN Fluorometers. In addition, we provide the dimensions for these wooden and PVC pieces in lumber units (i.e., inches) below, which typically serve as the standard unit of measurement for these types of materials and their related tools.

<table>
<thead>
<tr>
<th>Part</th>
<th>Approx. Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wooden &quot;4 x 4&quot; post (3 to 8 feet long)</td>
<td>$2 to $8</td>
</tr>
<tr>
<td>1-inch to 1½-inch PVC pipe (Schedule 40, 5 ft. long)</td>
<td>$2 to $3</td>
</tr>
<tr>
<td>¼-inch PVC pipe (Schedule 80, 4 to 6 inches long)</td>
<td>$1</td>
</tr>
<tr>
<td>¼-inch PVC pipe cap (Schedule 40)</td>
<td>$1</td>
</tr>
<tr>
<td>4x objective lens from a compound microscope</td>
<td>$10 to $15</td>
</tr>
<tr>
<td>LED flashlight (2,000 lumens with a convex lens)</td>
<td>$10 to $20</td>
</tr>
<tr>
<td>Sensitive Light Dependent Resistor (LDR)</td>
<td>$8 to $11</td>
</tr>
<tr>
<td>Digital Multimeter</td>
<td>$5 to $25</td>
</tr>
<tr>
<td>Jumper wires with alligator clips</td>
<td>$5</td>
</tr>
<tr>
<td>Pieces colored cellophane</td>
<td>$2 to $10</td>
</tr>
<tr>
<td>No. 6 cork stopper (to hold the LDR)</td>
<td>$1</td>
</tr>
<tr>
<td>Electrical tape or masking tape (one roll)</td>
<td>$1 to $3</td>
</tr>
<tr>
<td>Dark wood stain or flat black paint</td>
<td>$3 to $6</td>
</tr>
<tr>
<td>AA or AAA batteries (one four-pack)</td>
<td>$2 to $3</td>
</tr>
<tr>
<td>Total</td>
<td>$52 to $112</td>
</tr>
</tbody>
</table>

As with the OPN Scope,1 OPN Colorimeter,2 and OPN Spec,3 readers can make the wooden version of the OPN Fluorometer using hand tools. However, the instrument can be built much 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.), and these items should be available in the wood shops of most high schools, colleges, or universities. Also, anyone who is unfamiliar with these types of tools should make sure to review the Hazards section near the conclusion of this supplement given the significant dangers involved with this kind of equipment.

The Body the OPN Fluorometer

To build the body of the OPN Fluorometer (Fig. S4-1), we followed several of the same steps set forth in the Supporting Information for our OPN Colorimeter paper. Specifically, we used a 3½-inch tall section cut from a “4 x 4” post (which actually measures 3 ½ inches x 3 ½ inches), and we then drilled a series of holes into the top, front, and side faces to respectively hold: a lid or cap to cover the test tube; a 4x objective lens from a compound microscope (to focus the light beam) as well as a large PVC tube to hold the flashlight itself; and a cork stopper to hold a sensitive light dependent resistor (LDR) that we connect to a digital multimeter to take our readings. As with the OPN Colorimeter, we suggest using either “paddle” bits or “Forstner” bits to drill these holes since these two types bits are specifically designed to drill into wood. However, if using paddle bits, please take into account the long, pointed tips on the end to avoid accidentally drilling through the bottom or side of the block.

![Figure S4-1](http://pages.stolaf.edu/opn-lab/equipment/)

**Figure S4-1.** The “4 x 4” block that we use as the body for our wooden OPN Fluorometers. Note how the finished version on the right is stained on the inside to reduce the amount of reflective glare from the light beam.
For readers who are unfamiliar with these types of 4 x 4 posts, these pieces of wood can occasionally be found in the “spare” or “scrap” wood bin of a hardware store or lumber yard (often at a discounted price). Alternatively, readers can purchase new 4 x 4 posts from a lumber yard or other seller that are typically 8 feet long and cost around $9 each (at November 2016 prices, not including any taxes). These longer posts should make over 25 OPN Fluorometer bodies (more if each one is cut shorter than 3½ inches tall), which should be more than enough for a typical teaching lab.

As we explained in our OPN Colorimeter paper, before drilling the holes in the top, front, and side faces of the body for the OPN Fluorometer, we first mark the center of each face by using a pencil and a ruler to draw an “X” between the opposite diagonal corners of each side (Fig. S1-1). Once we have finished marking the center of each face, we then place the 4 x 4 block in a vice that is bolted to the platform of a drill press, and we begin to drill our respective holes.

We begin by drilling a small guide hole deep into the center of the top, front, and side faces of the 4 x 4 block, using a ⅛-inch diameter drill bit. We take this step so that we can line up subsequent drill bits with each guide hole as we go forward, which makes it easier to find the center of each face (especially since we will be drilling multiple holes with different diameters into several of the faces).

Then, we drill a 1⅛-inch diameter hole approximately ½ inch into the center of the top face. This hole will act as a “collar” that will help hold in place the PVC pipe and cap, which will later cover the test tube. Next, keeping the 4 x 4 block in the same position and using the same ⅛-inch guide hole to center our new drill bit, we drill a ½-inch diameter hole much deeper into the top face (roughly 2 to 2½ inches deep) to hold the test tube itself. Specifically, we use a VWR Disposable Culture Tube, No. 47729-572, which has a 13-mm or roughly ½-inch diameter (S1). As such, the hole for the test tube should end at least ½ inch from the bottom face, which should provide a thick enough base to support the test tube. In addition, readers may find it necessary to widen the hole for the test tube a bit using sandpaper or a wood file, so that the test tube will slide smoothly into its chamber. Also, if using a different
test tube with a larger (or smaller) diameter, readers will need to change the diameter of this hole accordingly.

Next, in the front face of the block, we drill a 2-inch diameter hole roughly ½ inch into the center of the face (using our earlier guide hole) to hold a large PVC tube for the flashlight. In particular, we typically to use an Outlite WT03 tactical LED flashlight in our OPN Fluorometers, and this light has a cylindrical head with a 37-mm (or nearly 1.46 inch) diameter. As a result, we use a 1½-inch Schedule 40 (white) PVC tube to hold this flashlight. For readers who are unfamiliar with these types of PVC tubes, the 1½-inch dimension refers to the inner diameter of the pipe. The outer diameter of the tube is roughly 1.9 inches, which is why we use a 2-inch drill bit for the initial hole. Of course, if using a different LED flashlight with a smaller head, a smaller PVC tube might be necessary, so readers may need to drill a hole with smaller diameter for this step (see Figure S4-3 below for some examples of these types of flashlights).

Then, we keep the 4 x 4 block in the same position and, using our ⅛-inch guide hole to center our next drill bit, we drill a ¾-inch diameter hole deeper into the front face (roughly 1¼ inches) until we pierce the hole that we previously drilled for the test tube (readers should feel the resistance on the handle of the drill press ease up as soon as they have broken into the hole for the test tube). This ¾-inch hole in the front face of the wooden block will hold a 4x objective lens for an AM Scope compound microscope (SKU No. A4X-V300), which has a ¾-inch diameter (Fig. S4-2, right). Readers, however, may find it necessary to widen this hole a bit using sandpaper or a wood file, so that the AM Scope objective lens will smoothly slide into place after it is wrapped in the piece of colored cellophane, which serves as its excitation filter. Of course, if using a different objective lens, readers may need to drill a larger (or smaller) hole to fit this piece.

Finally, in the center of the side face, we drill a ⅝-inch diameter inch hole roughly 2 inches into the center right face to hold a No. 6 cork stopper that has a maximum diameter of ¾ inches and will contain the LDR (Figs. S4-6A and S4-9). Again, readers should feel the resistance on the handle of the drill press ease up as soon as they have broken into the hole for
the test tube. With a ⅝-inch hole, the cork stopper will stick out of the side of the body, which makes inserting and removing the stopper much easier (Figs. S4-9 and S4-10). Of course, readers can choose to drill the hole for the cork stopper in either side of the OPN Fluorometer body. The choice is simply a matter of preference.

Next, we use a small paint brush to stain the inside of all of the holes that we previously drilled into the body (Fig. S4-1, right). Specifically, we use a dark (e.g., ebony) wood stain in order to cut down on the amount of glare caused by the unfinished wood (Fig. S4-1, right). In fact, we have found that it is very difficult to obtain useful readings with the wooden version of the OPN Fluorometer without this type of dark inner chamber for the test tube (likely due to the bright light reflecting off of the sides of the unfinished wood). Also, if readers do not have any wood stain available, they can use flat black paint instead. However, because paint can often sit on top of the wood after it dries (instead of sinking into the wood), it may be necessary to drill slightly larger holes for each piece. For this reason, we recommend using a dark wood stain (instead of paint) to coat the inside of the OPN Fluorometer, and readers can usually purchase small cans of dark wooden stain at a hardware store for around $5 each. Alternately, some highschool, college, or university woodshops may even have a supply of stain that instructors could use when building their OPN Fluorometers.

The Objective Lens

As with the 3D-printed version of the OPN Fluorometer (S1), we place a 4x objective lens from a compound microscope (Fig. S4-2) in the front face of our OPN Fluorometers to help focus the beam from our flashlight and increase its intensity. Among the lenses that we use are (i) a 4x objective lens from a used Carlsan microscope that we purchased on eBay for roughly $15 (one of the same lenses that we use with the OPN Scope);¹ and (ii) new 4x objective lenses by AM Scope (SKU No. A4X-V300), which can be purchased online at http://www.amscope.com/accessories/objective/4x-achromatic-microscope-objective-for-compound-microscopes.html for roughly $13 (at November 2016 prices, not including any taxes or shipping). Of course, readers can use lenses from other microscopes (including any old or broken microscopes that they may have at their schools or which they can find online).
However, one advantage of the AM Scope 4x objective lens is that its cylindrical end has a ¾-inch diameter, which fits fairly easily into the hole created by a standard ¾-inch drill bit.

![Figure S4-2. The 4x objective lenses that we use with our OPN Fluorometers: a 4x objective lens from a used Carl'san microscope that we purchased on eBay (left); and a new 4x objective lens made by AM Scope (SKU No. A4X-V300; right).](image)

**The Light Source**

As with the 3D-printed version of the OPN Fluorometer (S1), we typically use an Outlite WT03 or A100 tactical LED flashlight (Fig. S4-3, left side) as the light source in our OPN Fluorometers (the same models that we frequently use with the OPN Scope). Both lights have a convex lens, an adjustable head for focusing the beam, and three brightness settings – which, when combined, enable these flashlights to generate up to roughly 2,000 lumens when placed on the “high” setting (S1). However, we have found that the Outlite WT03 can become quite warm when left on for long periods of time, so readers should take special care to monitor this flashlight when it is in use (S1). Also, because the cylindrical head of the Outlite WT03 is smaller than the 1½-inch inner diameter of the PVC pipe that we use for our light tube, we typically wrap this flashlight with several layers of masking tape, so that it will fit smoothly into the PVC tube (for an example of this technique, see Figure S4-9 below). Alternatively, the head
of the Outlite A100 tends to fits snugly into a 1½-inch PVC tube, although readers will likely need to use a small block of wood to hold the handle upright and keep the beam balanced.

![Figure S4-3](http://pages.stolaf.edu/opn-lab/equipment/) 

**Figure S4-3.** Tactical LED flashlights that we use with our OPN Fluorometers. From left to right, the Outlite WT03, Outlite A100, UltraFire SkyWolfEye, and GeakLight models.

Of course, readers can use other LED flashlights in the OPN Fluorometer too, and we have found that several different brands work well, such as the UltraFire, SkyWolfEye, and GeakLight models (Fig. S4-3, right side). Importantly, these particular flashlights all have cylindrical heads that are between 26- and 30-mm (1.02- and 1.18-inches) in diameter. As a result, after being wrapped with a few layers of tape, they can fit smoothly into a 1- or 1¼-inch PVC tube that can be used in place of the larger 1½-inch PVC pipe (as described below).

**The Tube for the Flashlight**

To hold the Outlite WT03 tactical LED flashlight, we use a 6-inch long piece of 1½-inch Schedule 40 PVC pipe, which has an outer diameter of roughly 1.9 inches. Typically, readers can purchase these tubes in 5-foot lengths, so one pipe should make 10 flashlight holders. As with the OPN Scope and OPN Spec,¹ ² we hold this tube in place using a compression fitting –
specifically, we wrap the tube with a few layers of electrical tape, so that it fits snugly into the hole in the front face of the body of the OPN Fluorometer (Figs. S4-4 and S4-9).

Also, because the head of the Outlite WT03 has a smaller outer diameter than the inside of the PVC tube, we wrap this flashlight with several layers of masking tape until it fits more smoothly into the PVC pipe (for an example of this technique, see Figure S4-9 below). Given the length of the cylindrical head of this flashlight and the larger diameter of the PVC tube, readers will need to use many layers of tape to make the light fit snugly into the tube. For this reason, we typically use masking tape (instead of electrical tape) for this task since it helps to keep costs low. Readers can take the same approach with the UltraFire, SkyWolfEye and GeakLight flashlights as well since the heads of those flashlights will also need to be wrapped with tape to fit into their respective PVC tubes.

![PVC tubes and flashlights](image)

**Figure S4-4.** The PVC tubes that we use with our OPN Fluorometers. From left to right, a 1½-, 1¼-, and 1-inch diameter Schedule 40 PVC tubes, which will fit an Outlite WT03 flashlight, an UltraFire or SkyWolfEye flashlight, or a SkyWolfEye or GeakLight flashlight (respectively).

**The Cover for the Test Tube**

To cover the test tube, we use a 3¾-inch long piece of ¾-inch Schedule 80 (dark gray) PVC tubing and a corresponding Schedule 40 (white) pipe cap (Fig. S4-5). As explained in the Supporting Information for our OPN Colorimeter paper, we have
found that this particular set-up is able to sufficiently block out any ambient light in the room to allow for useful readings from the LDR and multimeter. Alternatively, readers can use a piece of $\frac{3}{4}$-inch Schedule 40 (white) PVC pipe. However, as with the wooden version of the OPN Colorimeter, readers should place a rolled up piece of black construction paper inside the tube to help block out the light. Finally, regardless of the type of PVC tube used, readers should wrap the bottom end of the pipe with a few layers of electrical tape (Fig. S4-5), so that it will fit snugly into the 1$\frac{1}{8}$-inch “collar” drilled into the top face of the 4 x 4 block, which serves as the body of the OPN Fluorometer.

![Figure S4-5. Making a cover for a small test tube using a length of Schedule 80 PVC pipe (dark gray) and a Schedule 40 PVC cap (white). (A) The unassembled pieces. (B) The assembled pieces. Note the use of electrical tape at the base of the PVC tube to provide a secure compression fitting when the cap is inserted into the hole on the top face of the body of the OPN Fluorometer.](image)

The Light Dependent Resistor (LDR) and Digital Multimeter

As with the 3D-printed version of the OPN Fluorometer (S1), we typically use as our detector a sensitive light dependent resistor (LDR) that is available at [www.digikey.com](http://www.digikey.com) for between $7 and $9, depending upon the quantity purchased (at November 2016 prices, not
including any taxes or shipping costs; Fig. S4-6A, showing a NSL-6910 photocell by Luna Optoelectronics, which has a 67-kOhm “dark” resistance). Because the leads of this LDR are rather fragile, however, we drill two small holes through a standard No. 6 cork stopper to hold this component (rather than trying to push the leads through the cork directly). Ultimately, we connect the LDR to a digital multimeter, using “jumper” wires with “alligator” clips on them (S1). Over the past year, we have bought several different multimeters online or at a regional hardware chain for between $5 and $25 (Fig. S4-6B), and each one of these models has worked well with the set-up.

Figure S4-6. The Light Dependent Resistor (LDR) and multimeters that we use with our OPN Fluorometers. (A) NSL-6910 LDR (or photocell) by Luna Optoelectronics mounted in a No. 6 cork stopper. (B) Cen-Tech, Gardner Bender, and Craftsman digital multimeters (from left to right).
Readers, of course, can use other LDRs or multimeters instead. However, if doing so, please conduct a sufficient number of pilot tests with the OPN Fluorometer to confirm that the set-up is working properly and generating expected results.

**The Cellophane Filters**

As with the 3D-printed version of the OPN Fluorometer (S1) and the wooden version of the OPN Colorimeter, we use pieces of colored cellophane for our excitation and emission filters in the OPN Fluorometer (Fig. S4-7). Typically, we cut out square pieces that are roughly 3-inches x 3 inches or approximately 8-cm x 8-cm ($l \times w$). We then wrap one piece of cellophane around the end of the 4x objective lens (to serve as the excitation filter) and the other piece of cellophane around the LDR after it has been placed into the cork stopper (to serve as the emission filter).

![Image of cellophane filters](image)

**Figure S4-7.** Making excitation and emission filters for the OPN Fluorometer using pieces of cellophane.

Of course, readers will need to pick the appropriate color of cellophane for each filter based on their particular application. For example, in the relative fluorescence assays described in the Supporting Information (S5), we used green and red cellophane as the excitation and emission filters (respectively) in the Rhodamine B experiment that we conducted and blue and green cellophane for the excitation and emission filters (respectively) in our Acridine Orange tests.
Also, like the cellophane filters for the OPN Colorimeter, these filters are not specific for a particular wavelength (e.g., 650 nm exactly). Nevertheless, they can still be used to generate useful data for instructional or educational purposes.

In addition, to help readers in conducting their own educational exercises or other experiments with the OPN Fluorometer, we have included in Figure S4-8 and Table S4-2 the transmission spectra for two popular packets of colored cellophane sheets (the Edukit and Hygloss brands) sold on Amazon for between roughly $6 and $8. These cellophane sheets are the same ones described in our OPN Colorimeter paper and the Supporting Information for the 3D-printed version of the OPN Fluorometer (S1), and so are the related figure and table. However, for the convenience of readers, we include the data on these products here, and we also summarize the protocols that we followed to obtain these results.

In particular, we began by cutting small rectangular pieces of cellophane (roughly 2 cm x 5 cm) from each sheet of colored cellophane. For each test, we taped each piece of cellophane to the side of an empty cuvette chamber in a commercial (Hitachi U-1100) spectrophotometer, which had been placed on the “Visible” setting. We then recorded transmittance values for each piece of cellophane between 400 and 700 nm (in 10-nm increments). In particular, we first “zeroed” the spectrophotometer using an empty chamber, then slid into place the test chamber with the colored cellophane, next recorded the corresponding transmittance value displayed on the digital readout, and finally repeated this process for the next wavelength. Once finished, we entered our data into a Microsoft Excel spreadsheet and graphed the results as scatter plots (Fig. S4-8).
Figure S4-8. Transmission spectra for different colors of Edukit (A and B) and Hygloss (C and D) cellophane sheets, which can be used as excitation and emission filters in the OPN Fluorometer.

We then reviewed the numbers to identify ranges of “useful” wavelengths for these two brands of colored cellophane sheets (Table S4-2). Specifically, for each brand, we used ranges with transmittance values of over 45% for the violet spectrum and over 60% for the blue through red spectra. As explained in our OPN Colorimeter paper, although these results were not identical to prior published findings, the ranges do appear to be similar.

<table>
<thead>
<tr>
<th>Color</th>
<th>Edukit Range (nm)</th>
<th>Hygloss Range (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Violet</td>
<td>400 to 440</td>
<td>400 to 480</td>
</tr>
<tr>
<td>Blue</td>
<td>400 to 510</td>
<td>400 to 520</td>
</tr>
<tr>
<td>Green</td>
<td>510 to 540</td>
<td>520 to 560</td>
</tr>
<tr>
<td>Yellow</td>
<td>540 to 700</td>
<td>510 to 700</td>
</tr>
<tr>
<td>Orange</td>
<td>580 to 700</td>
<td>570 to 700</td>
</tr>
<tr>
<td>Red</td>
<td>600 to 700</td>
<td>600 to 700</td>
</tr>
</tbody>
</table>

Assembling the OPN Fluorometer

Once all of the pieces of the OPN Fluorometer have been made, assembling the device is fairly simple. First, we wrap the 4x objective lens in the piece of cellophane that serves and the
excitation filter and the cork stopper containing the LDR in the piece of cellophane that serves and the emission filter (Fig. S4-9A). Then, we place these two components into their respective holes located in the front and side faces of the OPN Fluorometer (Fig. S4-9B). Next, after wrapping one end of the large PVC tube for the flashlight with a few layers of electrical tape, we place this tube into the front face of the OPN Fluorometer body (Fig. S4-9C). Note that it may be necessary to place a small block of wood underneath this tube for support (Fig. S4-D). Then, we place the smaller PVC pipe and cap, which serves as the cover for the test tube, into the hole in the top face of the OPN Fluorometer (Fig. S4-9C). Again, note that we wrap the bottom end of this PVC tube with a few layers of electrical tape in advance, so that it fits snugly into the top hole. Finally, we wrap the head of the LED flashlight with the appropriate amount of masking tape, so that it will fit smoothly into the large PVC tube (Fig. S4-9A). As with that PVC tube, it may be necessary to place a small block of wood under the handle of the flashlight to help steady the beam (Fig. S4-9D). Then, once the parts of the OPN Fluorometer have been assembled, the leads of the LDR can be attached to a digital multimeter, so that students can begin taking readings (see Figure S4-10 for an example of this set-up).

Figure S4-9. Assembling a wooden version of the OPN Fluorometer. (A) The unassembled pieces of the instrument. (B) Inserting the 4x objective lens and cork stopper into the body of the OPN Fluorometer. (C) Inserting the PVC tube for the light source and the PVC tube that covers the test tube into the body. (D) An assembled OPN Fluorometer.
Making Alternate Wooden Versions of the OPN Fluorometer

Readers should know that we did build several wooden versions of the OPN Fluorometer to hold a glass cuvette (instead of a small test tube). These designs were based on the various wooden versions of the OPN Colorimeter, where we used a ½-inch thick piece of plywood (which served as the cuvette chamber) glued together with other pieces of ¾-inch thick wood (which served as the surrounding body for the device). Even though we stained the inner chambers of these models with dark stain (as described above), we were not able to obtain consistent readings from these versions of the OPN Fluorometer using a glass cuvette. Also, despite numerous attempts at troubleshooting these models, we were not able to identify (or, more importantly, resolve) the issue(s) causing these inconsistencies or anomalies in our data. For this reason, we have not discussed any of these alternative designs for the OPN Fluorometer in this supplement.

On a related note, while readers can try building a wooden version of the OPN Fluorometer by gluing together individual pieces of ¾-inch-thick wood (similar to some of our OPN Colorimeter designs), we believe that, in the long run, it will be easier to use a solid 4 x 4 block of wood as the body for the OPN Fluorometer. For starters, once cut, a 4 x 4 block does not need to be sanded in order to have smooth faces and a level base. In addition, readers may find it easier to drill all of the respective holes into a solid 4 x 4 block of wood (instead of having to glue together the individual ¾-inch-thick pieces first, sand them flat, and then drill them). Finally, depending upon the size of the holes, some of the ¾-inch wooden pieces may splinter or crack as they are drilled (especially if the hole is sunk in from the side or top of a piece instead through its face). These cracks could further leave gaps in the wood, which would likely need to be closed (e.g., using wood putty) in order to prevent any ambient light from the room from striking the solution under examination during the experiment.

Hazards

As explained in the Supporting Information for other OPN instruments that we have made, hand and power tools are very dangerous, and the risks of injury associated with them are as significant as they are obvious. Consequently, readers should exercise a great deal
of caution and care when using any tools to build or assemble this instrument (including wearing the proper eye and ear protection).\textsuperscript{1-3} Also, if unfamiliar with these types of tools, we once again recommend that readers work with an experienced tradesperson for safety reasons.\textsuperscript{1-3} Alternatively, as with the OPN Scope,\textsuperscript{1} OPN Colorimeter,\textsuperscript{2} and OPN Spec,\textsuperscript{3} in some schools, a shop class could build these fluorometers for a science class as part of a semester project.

**Helpful Hints**

Given our experiences in developing and testing this version of the OPN Fluorometer, we provide the following helpful hints here for its use. Readers will likely notice that some of these suggestions as similar to those offered in our OPN Scope, OPN Colorimeter, and OPN Spec papers.\textsuperscript{1-3} We nevertheless repeat those recommendations here for the sake of convenience.

First, all of the helpful hints set forth in the Supporting Information on the 3D-printed version of the OPN Fluorometer (S1) are just as applicable to the wooden version. For example, as discussed above, we use compression fittings to hold in place the PVC tubes for the flashlight and test tube cover (Figs. S4-4, S4-5, and S4-9). Similarly, we use “jumper” wires with “alligator” clips on them to connect the leads of our LDRs to the probes on our multimeters (Fig. S4-10). Likewise, we regularly use removable mouting putty or poster tack to secure our wooden OPN Fluorometers to the table top in order to prevent them from being inadvertently bumped or jostled during and experiment (S1). Of course, readers could alternatively use Scotch “Extreme” Fasteners®, Velcro strips, or other adhesives to fix their OPN Fluorometers to wooden boards, which are then placed on the table top.\textsuperscript{2,3} This approach might even make the instruments easier to use with students (in addition to reducing the amount of time that it would take to set up and later break down a lab).
Figure S4-10. Connecting the probes of an inexpensive digital multimeter to the leads of a light dependent resistor (LDR) using jumper wires with alligator clips on them.

Similarly, as with the 3D-printed version of the OPN Fluorometer (S1), instructors may want to have their students tape their LED flashlights into place before starting an experiment, so that the light does not move while an assay is in progress (potentially rendering subsequent results incomparable to earlier ones).\(^2\) Instructors may also want to consider dimming or turning off the overhead lights during any lab exercises or in-class demonstrations with the OPN Fluorometer to cut down on the amount of bleaching that might occur due to the ambient light (S1) as long as this approach does not present any safety hazards for students or others.\(^3\)

Instructors should also have their students turn on their LED flashlights and place them in the PVC tube at least 10 minutes before beginning an experiment in order to given the light and the LDR a sufficient time to warm up (S1, S5). At the start of this period, we also suggest that students fill their test tubes with 4 mL of the solvent that they will be using, place those test tubes in their wooden OPN Fluorometers, and cover them with the PVC lid (S5). Then, students should record the resistance values associated with these “blank” solutions at regular
(e.g., 1-minute) intervals in order to track these changes and determine if the set-up has sufficiently stabilized (e.g., by showing less than a 1% change per minute). Instructors should also consider conducting a sufficient number of “test runs” before the actual lab activity in order to “trouble shoot” the exercise and have a sense of the type of data students will likely see (S1, S5).

Finally, before lab, instructors should discuss with their students how the resistance of the NSL-6910 photocell is inversely proportional to the intensity of the light striking it.\(^2,3\) As a result, any graphs created using data generated by the OPN Fluorometer will likely appear to be “flipped” versions of a typical concentration curve – i.e., these curves will decrease with concentration levels instead of increase (S1, S5). Alternatively, to generate a more typical looking calibration curve, instructors can have their students subtract the resistance values associated with their invidual fluorophore samples from their initial “blank” reading (S5), which should create curves that increase (and eventually level off) with concentration level.

References