S1: 3D Printing the Components of the OPN Scope

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 parts for the OPN Scope and OPN Drawer. We designed each of these components using the free version of DesignSpark Mechanical (RS Components, Ltd., Corby, Northamptonshire, UK), which is available at http://www.rs-online.com/designspark/electronics/eng/page/mechanical under the "Download DesignSpark Mechanical" heading. We have also included both the CAD and STL files for each part as Supporting Information (S2 and S3, respectively), so that readers can print or modify them as needed. In addition, we provide the general print dimensions and volumes for these parts in Table S1-1, so that readers can determine whether their 3D printers can accommodate the size of the components or whether other printing arrangements should be made.

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

Part	Dimensions (cm)	Volumes (cm^3)
OPN Scope		
Outer Shell	11.00 x 5.55 x 8.50	173.70, 20.20
Drawer	7.20 x 4.40 x 4.50	58.05, 6.10
Drawer Handle	0.95 x 0.95 x 8.30	5.25, 0.20
Light Tube	4.35 x 4.35 x 6.65	25.10, 1.50
Eyepiece Tube	3.30 x 3.30 x 5.70	19.85, 3.85
Mount Adapter	4.40 x 4.40 x 2.45	12.45, 1.65
OPN Drawer		
Drawer	7.20 x 4.40 x 4.50	44.75, 6.10
Drawer Handle	0.95 x 0.95 x 8.30	5.25, 0.20
Holders		
Ex. Filter	3.95 x 3.30 x 0.70	6.10, 0.70
Mirror (Rect.)	4.85 x 3.30 x 0.40	3.85, 0.85
Mirror (Circ.)	4.85 x 3.30 x 0.40	4.15, 0.85
Em. Filter	4.20 x 4.35 x 0.65	7.85, 1.95

We designed the outer shell of the OPN Scope (S2A, S3A) to be big enough to hold a drawer for an Olympus BX2-style fluorescence filter cube – one of the larger versions on the market (Fig. S1-1A, far left). The five OPN Scope drawers that we have designed to date (S2B01 – S2B05, S3B01 – S3B05) all have the same external dimensions and use the same handle (S2C, S3C). Thus, only the internal dimensions of the drawers need to change to accommodate a given filter cube (Fig. S1-1B). At present, we have designed drawers to hold the following types of cubes: Olympus BX2 (S2B01, S3B01), Zeiss Axio (S2B02, S3B02), Leitz/Leica Astroplan (S2B03, S3B03), Nikon Diaphot/TMD-style (S2B04, S3B04); and Leitz/Leica DMIRB (S2B05, S3B05) models (Figure S1-1A from left to right).

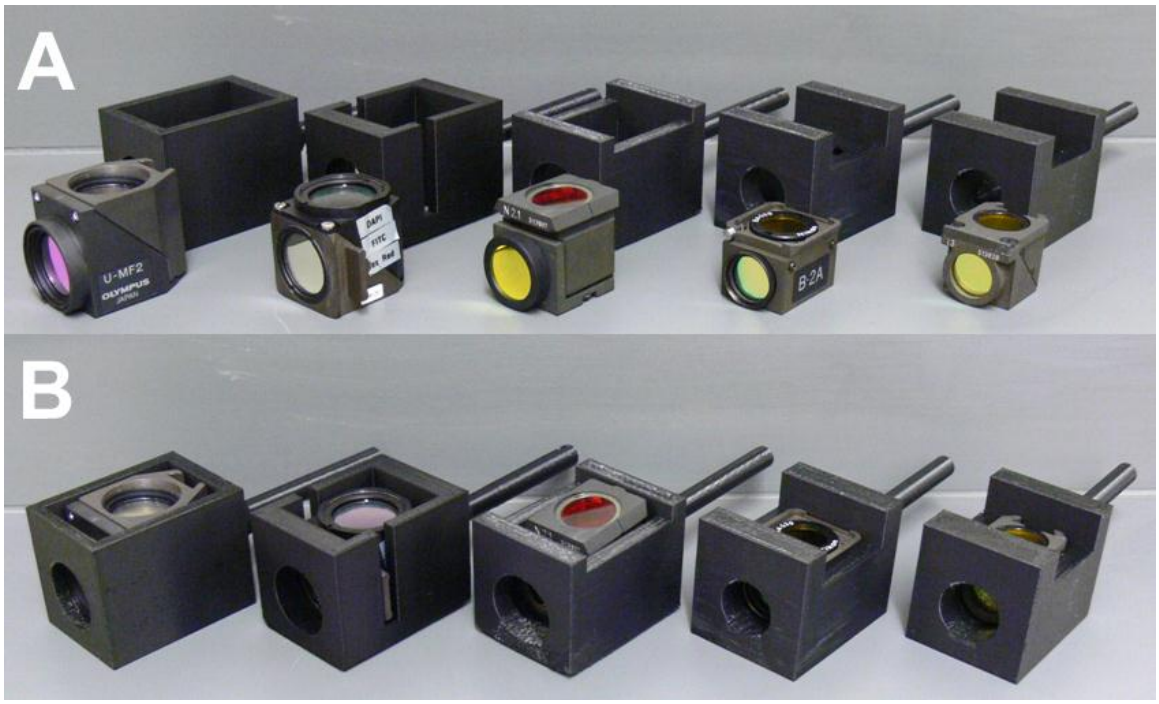

Figure S1-1. Examples of fluorescence filter cubes that will fit in the OPN Scope along with their corresponding drawers.

With respect to the other components of the OPN Scope, we designed the tube for the light source (S2D, S3D) to hold an Outlite WT03 tactical LED flashlight, which has a cylindrical head (and, thus, can easily slide into a circular tube). We further included a

4.75-mm diameter hole at one end of the tube, so that a 10-24 thumbscrew could be threaded through the opening to stabilize the flashlight.

We designed the tube for the eyepiece (S2E01, S3E02) to hold one from an Olympus CH-style microscope and the mounting adapter (S2F01, S3F01) to fit the same. We chose this microscope initially because it is used in several teaching labs at our school and generally costs between $350 (used) and $900 (new) online. We, however, have also designed eyepieces and mounting adapters to fit other compound microscopes, such as Nikon AlphaPhot-2 YSR, Nikon Eclipse E300, Zeiss AxioStar Plus, and Zeiss PrimoStar models (S2E02 – S2E05 and S3E02 – S3E05, S2F02 – S2F05 and S3F02 – S3F05, respectively) since these instruments are also used at our school.

Readers, however, can modify the CAD files for these components (or design their own) to create parts that would fit other compound light microscopes and their eyepieces. In so doing, however, please take care to maintain the proper tube length between the eyepiece and objective lens. Also, please know that some microscopes do not have removable eyepieces, such as the Nikon and Zeiss models described above. Nevertheless, in these instances, we have found that using an eyepiece from another microscope can suffice. For example, even though Olympus, Nikon, and Zeiss appear to have different tube lengths for their microscopes, we were able to bring images into focus between low (40x) and high (400x) magnification using an eyepiece from an Olympus CH-2 microscope placed in either its own tube (S2E01, S3E01) or the specific eyepiece tube for each Nikon or Zeiss model described above. Alternatively, readers can use a digital microscope camera in place of an eyepiece, and there are several inexpensive models on the market (although cameras with more MegaPixels will typically generate higher-quality images).

For the OPN Drawer (S2G, S3G), which also requires a handle (S2H, S3H), we removed large portions of the side walls, so that the dichroic mirror holder could easily

be slid into place (Fig. S1-2). We also created a chamber in the front of the drawer to hold the excitation filter, and we reduced the diameter of the hole in the front face to 15 mm to keep this filter in place. While readers can modify this dimension, we found no need to do so during our tests.

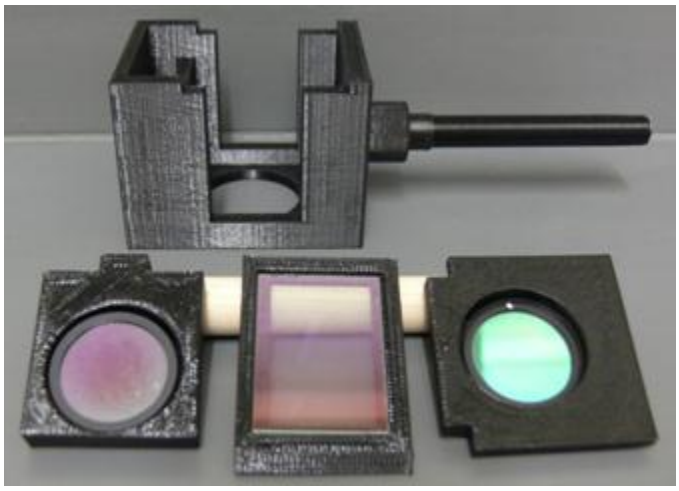

Figure S1-2. The OPN Drawer along with its holders for an excitation filter (left), dichroic mirror (middle), and emission filter (right).

The holders for the excitation and emission filters (S2I and S3I, S2L and S3L) can contain circular filters as big as 25-mm in diameter and 5-mm thick. The holders for the dichroic mirror can contain rectangular mirrors as big as 38 mm x 26 mm (l x w) and 1.0-mm thick (S2J, S3J) or circular mirrors with a maximum diameter of 25 mm and thickness of 1.0 mm (S2K, S3K). We chose these dimensions because they appeared to be the largest ones used in filter sets made by several prominent manufacturers. To 3D print components for smaller parts, readers need only change the corresponding internal dimensions in the supporting CAD files by using the "Pull" function in the free version of DesignSpark Mechanical.

STL Files and Test Prints

When generating the STL files for the OPN Scope and OPN Drawer in DesignSpark Mechanical (Export Options >> 3D Print (*.STL) >> Options), we found that setting the "resolution" to "custom" with a deviation of 0.05 mm and an angle of 0.1 degrees helped

to generate smooth curves in the resulting part. For even finer resolution, readers can enter values for the "facet maximum edge length" and "facet maximum aspect ratio," although these options create much larger STL files (some over 100 MB given the size of the components, which can end up crashing the program). Also, because different 3D printers may have different error tolerances, we recommend printing out a few test pieces first (e.g., a portion of a tube or part of the outer shell) to ensure that the above settings will generate the desired shapes and dimensions. These test prints should also indicate whether any dimensions in the CAD files need to be changed to accommodate a specific part.

3D Printing

We originally printed the parts for the OPN Scope and OPN Drawer on a Mojo 3D printer using QuickPacks of Black ABS build material and soluble support material (Stratasys, Ltd., Eden Prairie, MN). After a part had finished printing, we placed it in a WaveWash55 Support Cleaning System (Stratasys, Ltd., Eden Prairie, MN) until all of the support material had dissolved. We then rinsed each part in warm water and dried them before using them to assemble the OPN Scope. However, because the WaveWash55 solution is rather hot and has a pH of around 10, please read the Hazards section below before using the WaveWash55 system to clean any parts.

Of course, readers could use other 3D printers or filament instead. For example, we recently purchased a FlashForge Creator Pro 3D Printer (FlashForge USA, Rowland Heights, CA) and have successfully printed parts for the OPN Scope and OPN Drawer with 1.75-mm diameter SainSmart Black ABS Filament using MakerBot Desktop software and the Simplify3D slicing program.

Also, for those who do not have direct access to a 3D printer, there are websites, where STL files can be uploaded, printed, and shipped to you for a fee (which is usually based on the number of parts printed, the amount of filament used, and the shipping

costs involved). For example, in March 2016, we uploaded the STL files for the various components of the OPN Scope to www.3dprintingpricecheck.com, which estimated that it would cost between roughly $85 and $136 to print these parts in ABS plastic (high resolution) or strong and flexible black plastic, depending on the type of drawer involved using either the MakeXYZ or Shapeways printing websites (not including any taxes and/or shipping). Similarly, www.3dprintingpricecheck.com estimated that it would cost around $37 to print the components for an OPN Drawer that fit one filter set using MakeXYZ or Shapeways (again, not including any taxes or shipping). Thus, printing these parts along with the basic components of the OPN Scope should cost between $100 and $144 in total. For an exact quote, however, readers should upload their desired STL files to their preferred 3D printing website.

Hazards

As explained above, the solution used in the WaveWash55 Support Cleaning System is an alkaline one (pH 10), which is made by adding an Ecoworks tablet to an empty WaveWash55 container and then gently adding 7.5 liters (2 gallons) of warm tap water (away from the tablet) to avoid any splashes. Because the tablet itself as well as the subsequent solution can cause serious skin and eye irritation upon contact, please review both the WaveWash55 user guide and the Material Data Safety Sheet for the Ecoworks tablets before making or using the solution. Also, please wear the proper protective equipment (e.g., gloves, goggles, and a lab coat or heavy clothes) when preparing or working with the solution (we further use a pair of salad tongs to remove parts from the WaveWash55 container since the solution itself often becomes rather hot). Finally, please make sure to dispose of any used WaveWash solution properly since it is an alkaline mixture, which further contains dissolved support material (i.e., plastic). For example, we pour our used WaveWash fluid into large, shallow baking

pans and let the liquid evaporate over several days until only a malleable plastic remains, which we then scrape off and dispose of as waste.

Fluorescence Filter Cubes and Sets

In our OPN Scopes, we typically employ used fluorescence filter cubes, which can be purchased on eBay or other suitable marketplaces for used laboratory equipment (often for between $125 and $500 if buying a single-band cube, depending on the make and model). For example, the used cubes pictured in Figure S1-1A cost between $125 and $480 on eBay.

We further tested the OPN Drawer with the filters and dichroic mirror from a used Zeiss Axio series DAPI/FITC/Texas-Red cube that we purchased on eBay for $150 (Fig. S1-1A, second left). The excitation and emission filters measured 25 mm in diameter with thicknesses of 5.0 and 3.5 mm, respectively. The dichroic mirror measured 36 mm x 30 mm (l x w) with a thickness of approximately 1 mm. We then modified the internal dimensions of the three OPN Drawer holders to fit these parts and 3D printed them as described above.

The Light Source

After testing several different light sources, we found that tactical LED flashlights with a maximum brightness of 2,000 lumens (as well as a convex lens and adjustable head for focusing the beam) tended to generate the clearest and brightest fluorescent images. However, other LED flashlights may suffice. Three models that performed well during our tests were the Outlite A100, the Outlite WT03, and the Coast G20 inspection light (Fig. S1-3).

Figure S1-3. LED flashlights that we use with the OPN Scope: an Outlite A100 (left), an Outlite WT03 (middle), and a Coast G20 (right).

As our main light source, we use an Outlite WT03 tactical LED flashlight, which we purchased on Amazon for roughly $13 (Fig. S1-3, middle). We chose this model because it has three brightness settings (with a maximum of 2,000 lumens), a convex lens that enables the beam to be intensely focused, and a cylindrical head, so that the flashlight can easily fit into a 3D-printed circular tube.

Although the Outlite A100 (Fig. S1-3, left) also works well, this flashlight has a large ellipsoidal head, which does not easily fit into a cylindrical tube. Nevertheless, we have found that the beam from this flashlight can be shown directly into the hole leading to the excitation filter to induce fluorescence without the light tube needing to be present. In these instances, we typically use a ring stand and clamp to hold the flashlight in place.

We have also found that the Coast G20 inspection LED light (Fig. S1-3, right) can excite fluorescence in cells stained with Rhodamine B or Acridine Orange, although a more powerful light (e.g., a 2,000-lumens model) was needed to induce fluorescence in cells tagged with a fluorescent protein. Given its much smaller diameter, however, the Coast G20 light requires two cylindrical adapters (S2M, S3M) to fit into the larger light tube for the Outlite WT03 flashlight. Alternatively, readers can print a version of the OPN Scope that was designed only to hold the Coast G20 inspection light (S2N, S3N and S2O, S3O), which thus has a much smaller light tube. We include the general print dimensions and volumes for these three different components in Table S1-2.

Table S1-2. Approximate Print Dimensions (*l* x *w* x *h*) and Volumes (Build, Support) for Parts Fitting the Coast G20 Inspection Light

Part	Dimensions (cm)	Volumes (cm^3)
Adapters for Coast G20 (for Outlite WT03 tube)	7.65 x 7.65 x 1.55	26.25, 10.20
Outer Shell – Coast G20	11.55 x 5.55 x 8.50	172.10, 21.50
Light Tube – Coast G20	2.70 x 2.70 x 7.50	17.70, 8.40

Using the same March 2016 estimates from www.3dprintingpricecheck.com, printing the adapters for the Coast G20 inspection light would add between $6 and $12 to the prior printing costs on either MakeXYZ or Shapeways. Alternatively, printing the various components for the Coast G20 version of the OPN Scope (with just one drawer) would cost between $84 and $131, using either the MakeXYZ or Shapeways websites (not including any taxes or shipping costs).

Finally, given our experiences in testing and refining the OPN Scope and OPN Drawer, we provide some helpful hints for their use.

Helpful Hints for the OPN Scope

First, we use compression fittings when assembling the various parts of our OPN Scopes, which is why each hole was designed to be larger than its corresponding tube. While readers are free to alter these dimensions to ensure a tighter fit, we prefer to wrap

a few layers of electrical tape around the end of a tube to achieve the same result, especially in light of the variations in 3D printing error tolerances discussed earlier.

Second, when viewing fluorescent images, a flashlight may at times be too dim (or too bright) to obtain useful fluorescence. In these instances, readers can try using newer (or older) batteries, adjusting the position or angle of the light in the tube, changing the brightness setting, or adjusting the focus of the flashlight (assuming that it has these last two options). Alternatively, if using a fluorescent stain or dye, readers may want to try increasing (or diluting) its strength.

Third, some LED flashlights (especially those that lack a convex lens to focus the beam) can create a dark "halo" in the image or may light up only part of the field of view, and this latter effect can even occur with the Outlite WT03 when its head is not fully extended. In these instances, images can appear framed in darkness when viewed through the eyepiece or on a video screen. Similarly, part of a fluorescent cell can appear cut off if it happens to cross the edge of the halo or beam. If this occurs, it may be possible to correct for the effect by changing the position of the slide slightly or adjusting the position of the flashlight. However, using a flashlight with a convex lens that is fully extended should avoid this issue.

Fourth, if left on for prolonged periods of time in the light tube, the Outlite WT03 that we used became rather warm. As a result, consider turning off the light when it is not in use, which should also reduce the amount of fluorescent bleaching (i.e., the fading of a specimen) that can occur when it is bathed in excitation light for long periods.

Helpful Hints for the OPN Drawer

First, for those who are unfamiliar with the components of a fluorescence filter set, each piece should be marked in some way to indicate which direction the part should face. For example, the band around the excitation filter typically has an arrow on it,

pointing in the direction that the filter should face. Similarly, the band around the emission filter often has a dot or some other mark on it, indicating which side should face up. Likewise, the dichroic mirror frequently has a special coating on the side that should face the excitation light source. Readers should keep these markings in mind when placing the components of filter set into their respective holders and then into the OPN Drawer.

Second, if using the free version of DesignSpark Mechanical to change the dimensions of a holder to fit a particular filter or mirror, we recommend first changing the distances between the bottom "lips" that hold a part in place and then changing the distances between the "walls" that surround the edges of the filter or mirror. Otherwise, the part must be rotated in more complicated ways in DesignSpark Mechanical to access each side.

Third, some emission filters perform best when tilted at a slight angle in their filter cube (e.g., by 1º to 5º). If necessary, readers can achieve this result in DesignSpark Mechanical by selecting bottom "lip" that holds the emission filter in place, clicking on the "Move" button, and then rotating the structure by the desired angle.

Fourth, because the various components of a filter set, especially the dichroic mirror, are quite fragile, please exercise great care when handling them. As a result, instructors may want to assemble the parts of an OPN Drawer themselves, so that the filters and/or mirror are not accidentally scratched or broken.

Fifth, relatedly, we recommend having a dedicated OPN Drawer (or at least a dedicated set of holders) for each filter set. Not only does this approach make changing sets much easier, it further reduces the chances of damaging a filter or mirror by repeatedly moving it into and out of a holder or drawer.