

User Manual: 3D Printing Strategies to Obtain Optically Clear Cardiovascular Anatomical Geometries for Flow Characterization

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User Manual

3D printing strategies to obtain optically clear cardiovascular anatomical geometries for flow characterization

Below is a set of instructions for 3D printing an optically clear anatomical model. These instructions assume the user has produced or otherwise obtained the desired model geometry. We advise the user of this tool to follow all safety procedures provided by the manufacturer of the 3D printer in use as well as donning all necessary personal protective equipment.

This tool demonstrates how to design and print models with an emphasis on optical clarity using two commercially available 3D printers and print materials.

1. PolyJet style 3D printer (Objet260 Connex3; Stratasys, Eden Prairie, MN) using a stock clear resin (VeroClear RGD10) print material.
2. Stereolithography style printer (FormLabs Form 3/4) and a stock clear resin (FormLabs Clear V4).

While there may be other printers on the market which could be used to attain a similar result, the technique described in this tool have only been proven with the printers and materials listed. Please note that the mention of any commercial products and/or manufacturers does not imply endorsement by the FDA or the U.S. Department of Health and Human Services.

Supporting Documentation

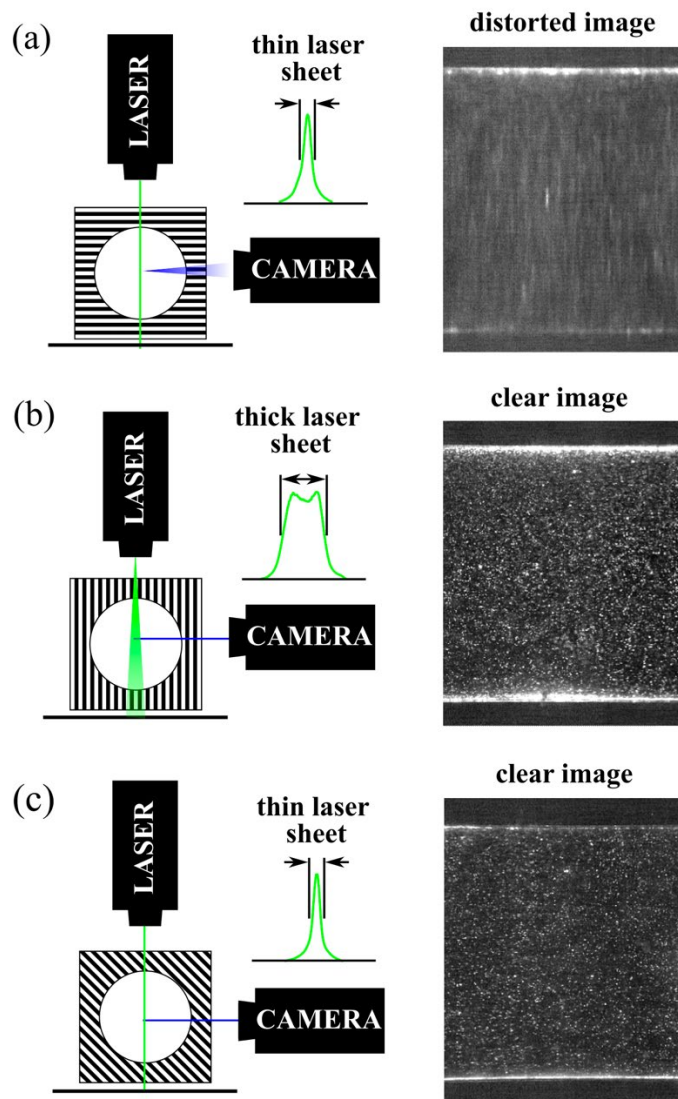
Print orientation

In 3D printing, print orientation refers to the physical orientation of the printed layers with respect to the region of interest. Print orientation can have numerous effects on final manufactured test section. Often 3D printers will have different spatial resolution in the plane of a single layer and than it does vertically through the printed layers (i.e. different resolution in the X and Y directions than it does in the Z direction). Similarly, for resin-based printers like those outlined in this tool, the resin may gather in certain locations depending on model geometry, e.g. well or troughs where resin may pool.

Beyond the general considerations, in the context of optical flow measurement or visualization print orientation is particularly important. Techniques like PIV and laser Doppler velocimetry, which require the use of a laser and are sensitive to image distortion, can be heavily influenced by print orientation. If instead the optical access is only being used for qualitative assessment of device placement, for example, the optical effects of print orientation are less influential.

If PIV or other similar methods are being used, print orientation may be critical to achieve adequate optical transparency. In the studies supporting this tool, orienting the print layers 45° offset from the plane of the camera and/or laser produced the highest image quality. Users may evaluate alternative orientations for their specific setup. The Figure below from Aycock et al. (2017, <https://doi.org/10.1007/s00348-017-2403-1>) demonstrates the effect on the quality of the image of the particles used for quantitative measurement. For those unfamiliar with PIV, the clarity of the image of the particles (the white dots in the black images show) is paramount to obtaining high quality data. Panels c) and d) demonstrate that particles are clearest with a 45° offset from the camera and the laser sheet is minimally distorted with 45° offset.

While this example is specific to PIV, this orientation also benefited camera-based flow visualization and device inspection in the studies supporting this tool.



Example orientations and resultant particle images. a) When the print layers are aligned with the camera there is significant distortion of the camera. b) When the laser is aligned with the print layers the laser sheet is too thick. c) When both the camera and laser are at a 45° offset from the print layers.

Print settings and model resolution

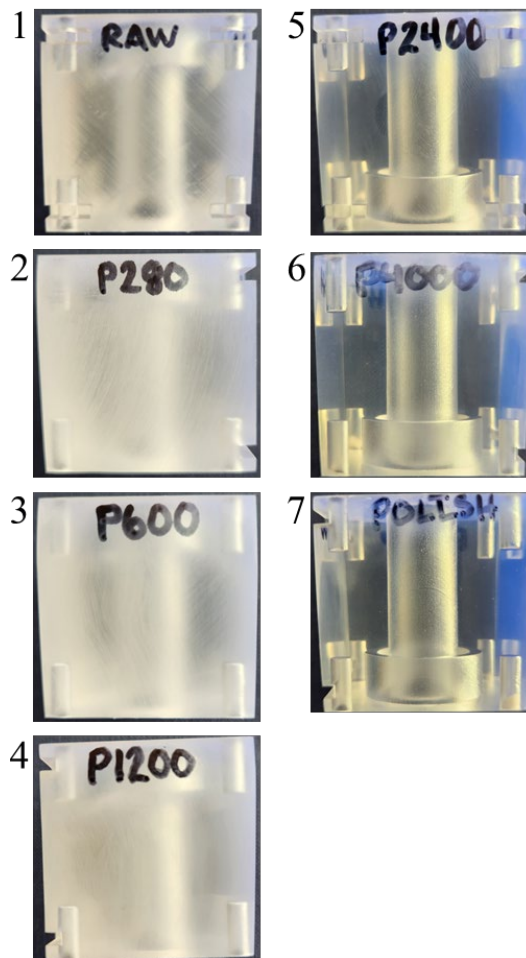
In 3D printing, there is often a trade-off between the speed and cost of printing and the spatial resolution of the print. In this work, printing the final design at the highest available printer resolution yielded the best optical clarity. Additionally, we suggest the input file created for 3D printing (e.g., an STL file) be the highest resolution available to increase number of faces and increase continuity on interior curved surface. Specifically, for printers referenced in this tool, we recommend the following:

1. Objet260 Connex3: stated resolution of 43 μm in X and Y, 16 μm in Z.
2. Formlabs Form 3: 25 μm in X and Y, up to 25 μm in Z for the clear resin used in these models.

Polishing external surfaces

To achieve a clear and minimally distorted view into the model lumen it is crucial to sand and polish the external surface of the printed piece. The external geometry of the test section used in this work included at least one flat surface for viewing and, if a laser or other illumination is being used, a second 90° offset flat surface.

To achieve a smooth surface, we recommend wet sanding starting with a relatively coarse grit sandpaper and moving toward progressively finer grits before using an abrasive polish. Changing sanding direction with each refinement in grit and sanding until lines from the previous pass are removed also helps to improve consistency in the surface finish. As shown in the figure below, we began with P280 grit and progressed through P600, P1200, P2400, and P4000. For a nearly mirror finish surface we then used an abrasive plastic polish with a polishing cloth (Novus P1200 Fine2 scratch remover polish). The samples in the figure were wet sanded and polished on a metallographic grinding and polishing wheel, but this process could be performed manually with water, sand paper, and a flat sanding block.



FormLabs external polishing stages including the wet sanding sandpaper grits and the polishing steps to achieve optical transparency.

Assessing internal surface quality and geometric accuracy

Surface roughness is important to characterize when performing flow visualization studies as roughness could trip laminar flow to transitional or turbulent flow earlier depending on the relative roughness (surface roughness/geometry diameter) and Reynold's number. Moreover, the internal surface quality can have a significant effect on the optical clarity of the model. The threshold roughness for the geometry of interest can be estimated using established fluid mechanics relationships (e.g., a Moody diagram). Then, the geometry surface roughness can be minimized to within the threshold via three potential methods. These three methods have the additional impact of effecting the geometric accuracy related to dimensional accuracy and faithfully printing curvature and corners. First, the STL surface quality can be improved to reduce faceting that can be visible in 3D prints by increasing the resolution of the triangulation or using different STL surface generation software other than the standard CAD software conversion. Next, changing the print orientation and increasing the print resolution can reduce surface roughness. Finally, for printers where internal surface polishing is required for optical

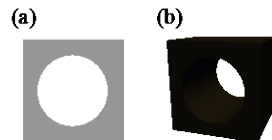
transparency (Stratasys Objet260 Connex3), the internal sanding and polishing method can be optimized to reduce roughness. Surface roughness can be quantified with an optical profilometer or high resolution microscopy that provide surface roughness characterization.

Matching index of refraction with working fluid

After optimizing the printing procedure and polishing the external geometry surface, a working fluid with an elevated index of refraction (IOR) is developed to reduce and eliminate distortions due to either the internal unpolished surface or curvature. Sodium iodide is the most common additive to increase the refractive index of a working fluid. Also, a small quantity of sodium thiosulfate can be added at the end of making the fluid to reduce discoloration caused by sodium iodide. The working fluid and its “recipe” to match the refractive index of a 3D printed part is dependent on the application. For example, if a Newtonian blood analog is necessary for cardiovascular applications, the working fluid will be comprised of glycerin, water, and sodium iodide that is incrementally adjusted by testing with a viscometer (or rheometer) and refractometer until the required viscosity and IOR is achieved. If only the IOR is important, the working fluid consists of deionized (DI) water and incremental additions of sodium iodide until the IOR of interest (checked with a refractometer) is achieved. Working fluid was stored in an amber bottle with a sealed lid to prevent color changes from light exposure and oxidation.

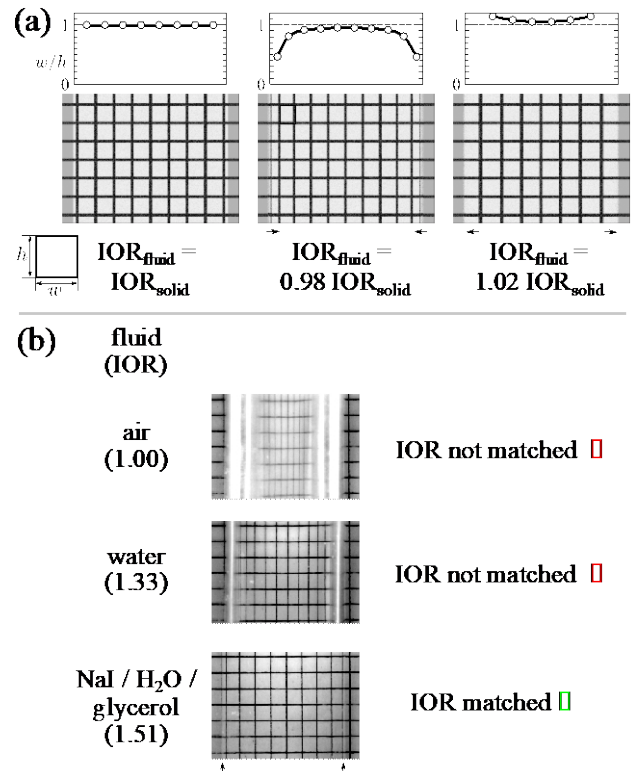
To determine the IOR of the 3D printed material, there are two potential methods. The first is a grid test outlined in Aycock et al. (2017), where incremental quantities of sodium iodide are added to the working fluid until the IOR is achieved. To perform the grid test:

1. 3D-print a test cube with a cylindrical hole through the central axis with the optimized, final print method. Polish the test cube by wet sanding with increasing grit size and polish.
2. Measure the IOR of the first batch of working fluid using a refractometer to know the starting IOR. Use a rubber stopper on one end of the test cube, fill the cube with the working fluid, and cap with a second stopper.
3. The test cube is placed on top of a 2mm square grid and an image is captured.



4. The width and height of randomly sampled squares are measured and the ratio of w/h is calculated.
 - a. If $w/h=1$, then the fluid refractive index is correct.
 - b. If $w/h < 1$, the fluid refractive index is too low
 - c. If $w/h > 1$, the fluid refractive index is too high
5. If the IOR is too low, incrementally add sodium iodide and repeat the above steps until $w/h=1$ and you have a measure via a refractometer of the fluid IOR.
6. If the IOR is too high, incrementally add DI water and repeat the above steps until $w/h=1$.

A second method to measure IOR is optical coherence tomography (OCT). OCT is a noninvasive method that directs light at a sample surface and measures the reflected signals, visualizing the cross section of a transparent material. This allows for the IOR to be quantified by calculating the ratio between the measured optical thickness and the physical geometric thickness. To obtain optical thickness, each OCT system needs a unique calibration between pixels and a known distance that is measured in air¹. Finally, the IOR of the 3D printed sample is measured by printing a thin sample (2-3mm thick), polishing using the optimized procedure, and the measuring the IOR using the OCT protocol unique to each system.



Technical Limitations

The printers and print materials used in this RST produce functionally rigid components. For some applications in which replicating the structural compliance of blood vessels is important, a compliant physical phantom may be desirable. As of the date of publication, no 3D print materials evaluated in this work demonstrated both the optical characteristics described herein and structural compliance comparable to that of a blood vessel wall.

¹ Bouma BE, de Boer JF, Huang D, Jang IK, Yonetsu T, Leggett CL, Leitgeb R, Sampson DD, Suter M, Vakoc B, Villiger M, Wojtkowski M. Optical coherence tomography. Nat Rev Methods Primers. 2022;2:79. doi: 10.1038/s43586-022-00162-2. Epub 2022 Oct 13. PMID: 36751306; PMCID: PMC9901537.

The size of each anatomical model is limited to the size of the print volume for each 3D printer. Depending on the printer and the desired model this may require the model to be printed in a series of components and assembled. For the printers detailed here the print volumes are:

1. Objet260 Connex3: 25.5 x 25.2 x 20.0cm
2. Formlabs Form 3: 14.5 x 14.5 x 19.3cm

In most cases, if the recommendations outlined herein are followed there will not be a need for polishing the internal surfaces of the model. However, there may be some cases in which the texture of the internal surface significantly influences the experiment, measure, or phenomenon in question, e.g. if tripping to turbulence is of great concern. In these cases, additional steps will need to be taken to polish or otherwise change the internal surface. Broadly speaking this involves printing the model in two halves, polishing the internal surfaces, then sealing and attaching the two halves. A polishing method similar to the one described for external surfaces was found effective for internal surfaces in the studies supporting this tool.

The spatial resolution offered by the 3D printers featured in this tool is supported imaging of the cardiovascular anatomical models evaluated in this work. However, these printers and the other methods described are likely not suitable for the smallest vessels or other micro-scale applications. For these cases other manufacturing techniques are likely more suitable.

Lastly, the print materials referenced in this RST were not evaluated for biocompatibility in the studies supporting this tool. In all experiments described, these materials were used with water or blood analog fluids only.