

Updated designs and detailed assembly instructions can be found at:
<https://morgridge.org/research/labs/fablab/designs/a-3d-printable-device-for-non-invasive-neuroimaging-of-adult-zebrafish/>

Labels referred to in the following instructions reference Haynes et. al., Journal of Cell Science 2026 Supplemental Figure 1, or Figure 4 of the website linked above.

Preparing and assembling the Standard and Multi-Size chamber

- 1. Deburr the prints.** Sand all printed parts so they are free of burrs and surface imperfections that would prevent pieces from mating correctly.
- 2. Chase the holes.** 3D printed holes are frequently undersized, tapered, or irregular. Chase all holes (H.1–H.5) with a drill bit matching the specified hole diameter before tapping. Chase H.3 to give a sliding fit on the dowel pins – a 2 or 2.1mm drill bit can be used. The pins are 2mm, so a slightly larger bit can be used to improve glide if necessary.
- 3. Tap the threaded holes.** Tap according to the diameter and thread pitch of each hole:

Hole Function	Tap/Chase
H.1 Inflow and outflow ports	1/16-27 NPT
H.2 Clamp support screws	#4-40 UNC
H.4 Thumbscrew	M3 x 0.5
H.5 Set screws	M2 x 0.4

Only the portion of H.4 that passes through the moving piece of the support needs to be tapped. These specifications are also given in the Supplemental Figure 1 legend of Haynes et al.

4. Modify the thumbscrew. Cut a 0.5 mm wide groove into the thumbscrew [P.4A], starting 3.5 mm from the collar and running away from the collar. The shaft diameter at the base of the groove must be 3/32" so that a matching external retaining ring [P.4B] can be seated in the groove to hold the thumbscrew in place.

5. Install the magnets. Attach neodymium magnets [P.6, P.7] with a non-toxic, water-safe epoxy (Water Weld) into the cylindrical indents on the bottom of the outer reservoir [H.6] and on the top of the tabs of the inner reservoir [H.7]. Dry-fit the two sets of magnets first to confirm correct magnet orientation before setting them in epoxy. Once seated, cover each magnet with an additional thin layer of epoxy to protect it from corrosion, and allow the

epoxy to cure fully per the manufacturer's instructions before exposing the chamber to water.

6. Install the dowel pins. Place the dowel pins [P.3] through H.3, where they act as rails for the adjustable intubation tube support (see Figure 2). Secure them with set screws [P.5] in the side holes [H.5].

7. Fit the tubing connectors and support screws. Install clamp support screws in the tubing support assembly clamp [P.2]. Fit the tapped inflow and outflow holes [H.1] with threaded-to-barb connectors sized for 1/16" ID tubing. Affix a blunt-end 20G needle to a male Luer-lock-to-barb connector and mount it in the intubation tubing support [P.9A, P.9B]. All tubing adaptors are sized for 1/16" ID tubing.

8. Leak test. Before using the chamber with an animal, fill the inner reservoir, run the pump at the working flow rate, and check the threaded fittings and the overflow reservoir for leaks over at least 15–30 minutes.

The full imaging setup can then be assembled as shown in Supplemental Figure 4 in Haynes et al 2026, or Figure 3 on the website.

Preparing and assembling the Simplified Chamber

STL files for this version of the design can be found at <https://morgridge.org/research/labs/fablab/designs/a-3d-printable-device-for-non-invasive-neuroimaging-of-adult-zebrafish/>

1. Deburr the prints. Sand all printed parts so they are free of burrs and surface imperfections that would prevent pieces from mating correctly.

2. Chase the holes. 3D printed holes are frequently undersized, tapered, or irregular. Chase all holes (H.1,H.2) with a drill bit matching the specified hole diameter before tapping.

3. Tap the threaded holes. Tap according to the diameter and thread pitch of each hole:

Hole Function	Tap/Chase
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H.1 Inflow and outflow ports	1/16-27 NPT
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H.2 Clamp support screws	#4-40 UNC
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4. Install the magnets. Attach neodymium magnets [P.6, P.7] with a non-toxic, water-safe epoxy (Water Weld) into the cylindrical indents on the bottom of the outer reservoir [H.6]

and on the top of the tabs of the inner reservoir [H.7]. Dry-fit the two sets of magnets first to confirm correct magnet orientation before setting them in epoxy. Once seated, cover each magnet with an additional thin layer of epoxy to protect it from corrosion, and allow the epoxy to cure fully per the manufacturer's instructions before exposing the chamber to water.

5. Fit the tubing connectors and support screws. Install clamp support screws in the tubing support assembly clamp [P.2]. Fit the tapped inflow and outflow holes [H.1] with threaded-to-barb connectors sized for 1/16" ID tubing. Affix a blunt-end 20G needle to a male Luer-lock-to-barb connector and mount it in the intubation tubing support [P.9A, P.9B]. All tubing adaptors are sized for 1/16" ID tubing.

6. Leak test. Before using the chamber with an animal, fill the inner reservoir, run the pump at the working flow rate, and check the threaded fittings and the overflow reservoir for leaks over at least 15–30 minutes.

The full imaging setup can then be assembled as shown in Supplemental Figure 4 in Haynes et al 2026, or Figure 3 on the website.



