



Nov 20, 2025

Mounting larval zebrafish (*Danio rerio*) for upright light sheet microscopy

DOI

<https://dx.doi.org/10.17504/protocols.io.ewov11br7vr2/v1>

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Protocol Citation: Chad Hobson, Lisa K. Randolph, Miray Girguis 2025. Mounting larval zebrafish (*Danio rerio*) for upright light sheet microscopy. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.ewov11br7vr2/v1>

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Protocol status: Working

Created: November 11, 2025

Last Modified: November 21, 2025

Protocol Integer ID: 232093

Keywords: zebrafish, mounting, Light sheet microscopy, larval zebrafish for upright light sheet microscopy, mounting larval zebrafish, upright light sheet microscopy this protocol, mosaic microscope, upright light sheet microscopy, mounting larval, larval zebrafish, portion of the specimen, specimen

Abstract

This protocol is for mounting larval zebrafish for upright light sheet microscopy. The protocol is generalized and may need to be adjusted slightly depending on the age and portion of the specimen of interest. The description here is specifically for mounting on the MOSAIC microscope (<https://www.aicjanelia.org/mosaic>; <https://www.biorxiv.org/content/10.1101/2025.06.02.657494v1>), however it can be readily adapted for similarly designed instruments.

Attachments



[20deg.jpt](#)

181KB



[20deg.stp](#)

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[40deg.jpt](#)

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Materials

Reagents

- UltraPure Low Melting Point Agarose (ThermoFisher 16520050)
- Poly-L-lysine hydrobromide (PLL) (MP Bio 02150177-CF)
- Borax (Sodium tetraborate) (Sigma-Aldrich 221732)
- Boric Acid (Sigma-Aldrich B7660)
- 200 Proof Ethanol (Decon Labs 2701)
- MilliQ Water
- Hydrochloric Acid (HCL) (Sigma-Aldrich 258148)
- E3 Medium (protocol available here: <https://cshprotocols.cshlp.org/content/2011/10/pdb.rec66449>)

Equipment

- 25 mm round #1.5 coverslips (Warner Instruments 64-0734)
- Custom 3D-printed agarose molds (design files available in the protocol attachments)
- Strainer
- Sonicator
- pH meter
- Paintbrush (Ted Pella 11806)
- Plastic transfer pipette
- Vortex mixer
- Heat block
- Glassware
- Hot/stir plate
- Microwave
- [Vacuum filter
- 1.5 mL Eppendorf tubes
- 10 cm plastic dishes
- Parafilm
- Fluorescence Stereoscope
- Oven

Solution Preparation

1 Tricaine

1. Stock solution - 4 g/L diluted in filtered fish water (E2, E3, etc.) stored in 40 ml aliquots at -30°C.
2. Working concentration - Dilute stock solution 1:40 to 100 mg/L in filtered fish water (E2, E3, etc.).

2 0.1 M borate buffer (1 L of buffer in a 2 L beaker)

1. Mix 3.1 g boric acid, 4.8 g borax, and 750 mL MilliQ water in a beaker.
2. Place beaker on a hot stir plate with a stir bar at 40°C for 7 hours to dissolve, cover the beaker with foil (you can stir at room temperature overnight).
3. Adjust pH to 8.5 using HCl and adjust final volume at the same time.
4. Filter the solution through a vacuum filter.
5. Store at room temperature.

3 0.5 mg/mL poly-L-lysine (PLL) solution (2 mLs)

1. Mix 10 mg poly-L-lysine hydrobromide in 2 mL of 0.1 M borate buffer.
2. Vortex thoroughly.
3. Store at 4°C.

4 Low-melt agarose solution (3% and 0.8%)

1. For 0.8% solution, mix 0.4 g low-melt agarose in 50 mL E3 medium (or preferred medium).
2. For 3% solution, mix 1.5 g low-melt agarose in 50 mL MilliQ water.
3. Microwave until agarose is fully dissolved and the solution is clear.
4. Aliquot into 1.5 mL Eppendorf tubes.
5. Place several aliquots onto a heat block at 42°C, store the rest of the aliquots at 4°C.

Note: It is more cost effective to use standard melting point agarose for the 3% solution rather than low-melt agarose. However, this means that they cannot be kept on the same heat block at 42°C as the standard agarose will need to be kept at a more elevated temperature to maintain liquid form.

Note: Low-melt agarose for mounting should be prepared at least several hours in advance or ideally the day before imaging to allow it to fully cool to 42°C before using it with larvae.



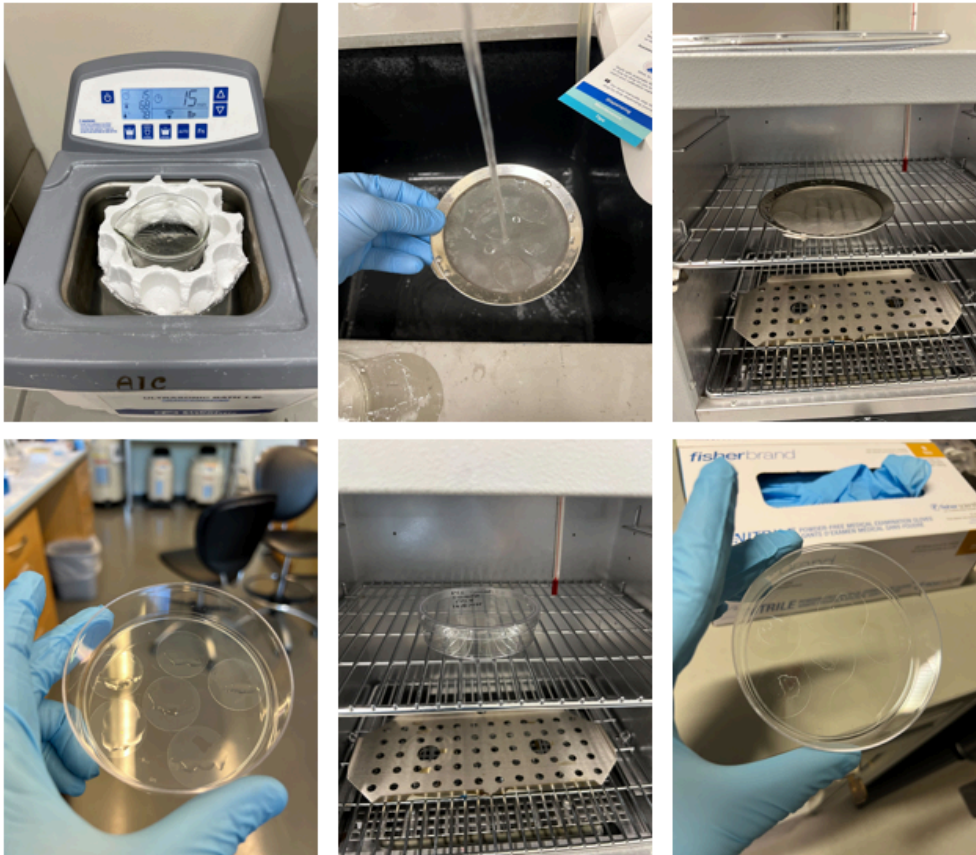
Agarose solutions before (left) and after (right) microwaving.

Sample Mounting

5 Coverslip Preparation

1. Sonicate coverslips in 200 proof ethanol for 15 minutes.
2. Strain out ethanol and rinse thoroughly with MilliQ water.
3. Dry coverslips in the oven at 65°C until fully dry.
4. Store coverslips in a 10 cm plastic dish sealed with parafilm.
5. When ready for mounting, pipette 10–15 μL of PLL solution slightly off-center onto a glass coverslip.
6. Spread PLL with a pipette tip to create a thin film covering approximately two-thirds of the coverslip.
7. Dry PLL by incubating coverslips in an oven for approximately 30 minutes at 65°C.
There should be a visible ring remaining where the PLL dried.

Tip: Before drying the coverslips in step 3, make sure that no coverslip is lying on top of another. If they are, the coverslips will stick together once dried.



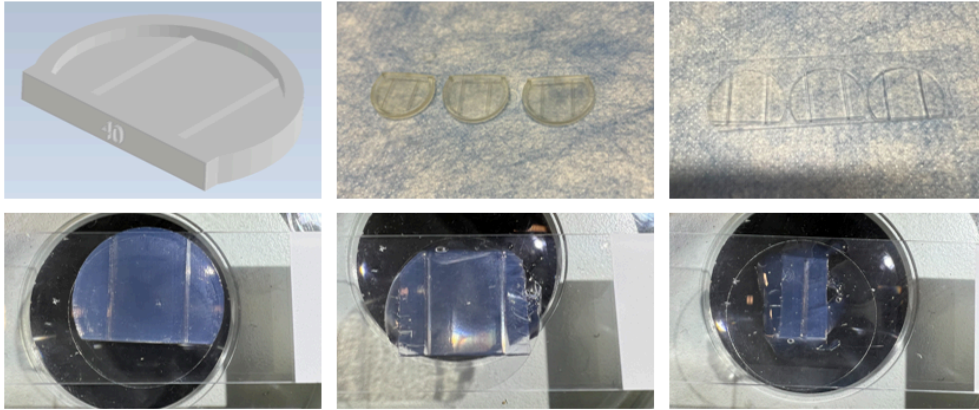
Sonicate (top, left), rinse (top, middle), dry (top, right), PLL coating (bottom, left), dry (bottom, middle), completed (bottom, right).

6 Mold Preparation

1. Use a transfer pipette to distribute 3% agarose into sample mounting molds, allow to solidify for 1-2 minutes. Do not overfill the molds.
2. Remove solidified agarose from the mold and place onto a PLL-coated coverslip such that the round edge of the mold aligns with the edge of the coverslip. Be sure to place the mold over the area covered with PLL.
3. We have experienced these molds periodically moving from the coverslip surface. To address this, there are 2 slight modifications we have found to be successful.
 - Option 1: Add a thin layer of molten 3% agarose to the edges of the mold to seal it to the cover slip. Ensure that the agarose does not fill the channel or rise above the top of the mold.
 - Option 2: Before placing the mold on the coverslip, trim the mold to include just a square region around one of the channels. Place the cut agarose mold on the PLL-coated surface. The mold should be off-center, allowing space for the sample holder to clamp the coverslip. Add a thin layer of molten 3% agarose to the edges of the

mold to seal it to the cover slip. Ensure that the agarose does not fill the channel or rise above the top of the mold.

Tip: During step 1, if the molds do become overfilled a razor blade may be used to scrape away excess agarose from the mold leaving a smooth surface.



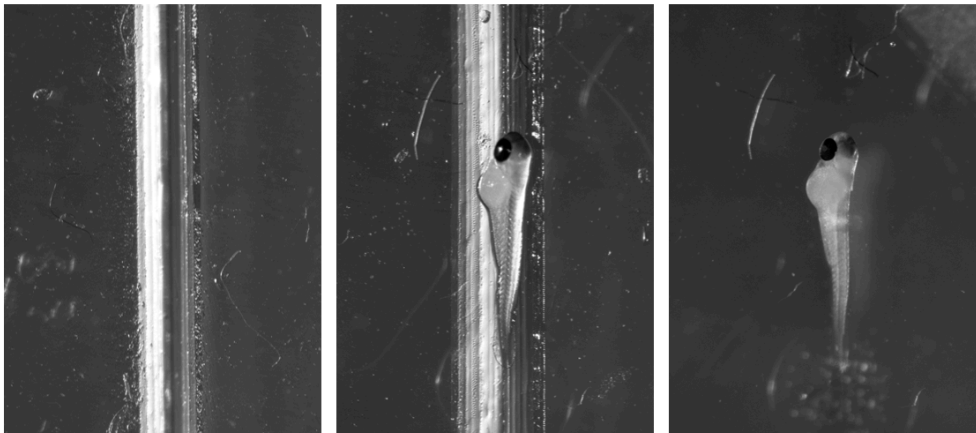
3D design of the mold (top, left), 3D printed molds (top, middle), 3% agarose molds (top, right), agarose mold on coverslip (bottom, left), sealed with 3% agarose (bottom, middle), and trimmed and sealed with 3% agarose (bottom, right).

7 Fish Mounting

1. Use a plastic transfer pipette to place larval zebrafish on top of the channel in the mold, transferring the smallest amount of water possible. The head of the fish should be pointing away from where the sample holder will be.
2. Use a small paintbrush to gently rotate the fish with the left side facing up such that it lays along the channel wall. Different molds may be used to create different angles at which the fish may be positioned.
3. Once the fish is positioned, remove as much water as possible using a pipette. Immediately replace the water with molten 0.8% low-melt agarose. Add just enough to fully cover the larvae ($< 50 \mu\text{L}$).
4. Once the low-melt agarose is added, quickly re-position the larvae as needed using the paintbrush.
5. Allow agarose to solidify for 1-2 minutes.
6. Place coverslip into the sample holder.
7. Place the sample holder onto the microscope and image.

Note: Depending on the developmental stage of the larva, it may be advantageous to first anesthetize the fish prior to moving it onto the agarose mold. In addition, it may be

necessary to dilute tricaine into the 0.8% agarose prior to covering the fish to maintain anesthesia during the mounting process.



Empty channel (left), zebrafish larvae mounted in the channel (middle), zebrafish larvae mounted in the channel and covered with agarose (right).