# Adult Zebrafish Imaging SOP

*All work should be done in accordance with local laws and regulations for your area, and under an established animal protocol.*

Preparation of Microscope Room

1. Turn on heat block to high to melt agarose and thaw tricaine aliquots.
    a. Turn heat block to low temperature (between 37 and 42 degrees C) after agarose is melted!
2. Prepare microscope by starting lasers and opening software.
3. Use Caviwipes to sanitize any room surfaces that will come in contact with animal containers or materials. Sanitize microscope stage or associated contact equipment with 70% ethanol. Use wipes for application, do not spray liquid directly onto equipment. Lens cleaner and lens paper ONLY should be used to clean lenses. Record sanitization on log sheet.
4. Fill clean reservoir with fresh system water with 150-170mg/L of MS-222 anesthetic.
    a. MS-222 aliquots are stored at -20°C with expiration date marked.
    b. MS-222 stock is made at 10mg/mL

    **Tricaine Dosage Guide**

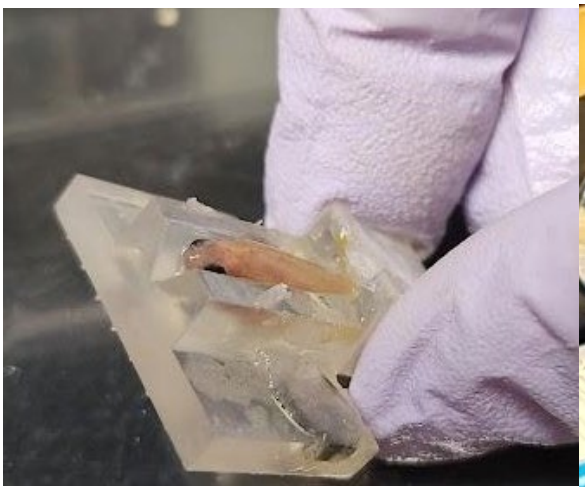
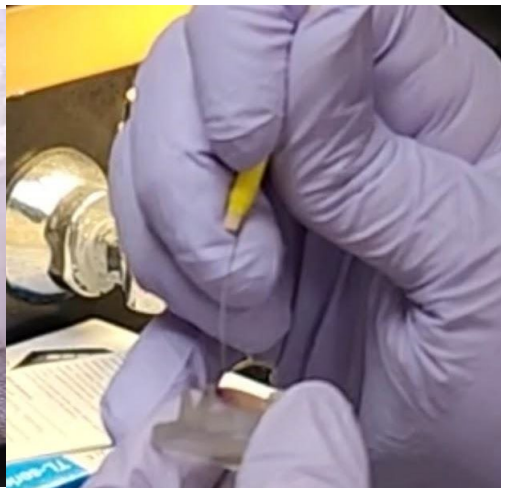
| Purpose | Final Concentration (mg/L) | Amount of 10mg/ml Tricaine to add to System Water |
|---|---|---|
| Maintenance (low) | 150 | 22.5ml to 1.5L water |
| Maintenance (med) | 160 | 24ml to 1.5L water |
| Maintenance (high) | 170 | 25.5ml to 1.5L water |
| Induction | 180-200 | 9-10ml to 500ml water |
| Euthanasia | 300-500 | 7.5ml-12.5ml to 250ml water |

    c. Note: Casper fish are more resistant to anesthesia, so monitor fish for signs of arousal. If fish is waking up after initial induction, increase tricaine concentration by adding 1ml at a time to the intubation reservoir until sedation is achieved, in accordance with above table. This is very dependent on the size of the fish. Exercise caution and closely monitor the fish.
5. Turn on reservoir heater.
6. Turn on reservoir aerator.
7. Insert chamber on microscope stage, taking care to move any objectives out of the way prior.
8. Hook up clean inflow tubing (connecting from reservoir to imaging chamber)
9. Hook up clean outflow tubing (connecting from reservoir to outflow reservoir)
10. Insert temperature probe into holding slot on imaging chamber, turn on temperature monitor.

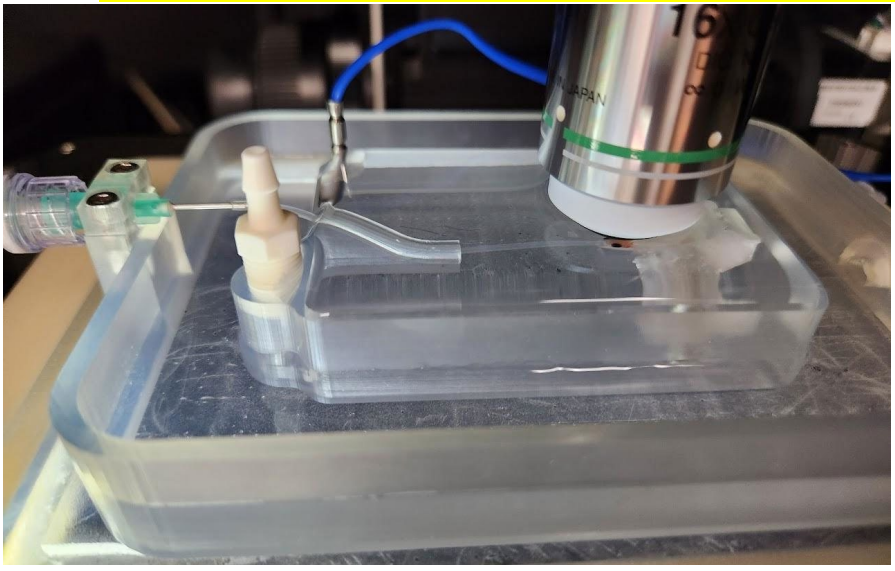
11. Turn on pump, set to 26.8rpm (6mL/min). Allow imaging chamber to fill with water from reservoir by briefly turning pump to the "fast forward" setting indicated by the double arrows, which will run the pump at its fastest speed. Return pump to 26.8rpm before mounting fish. Monitor water temperature and allow chamber and water in the chamber to reach desired temperature (~28 degrees C). Take care not to accidentally reverse pump direction (controlled by circular arrow button).
12. Attach desired objective onto the scope, ensuring that the stage is low enough to provide ample clearance for the objective to avoid damaging the objective or the animal. Prepare the microscope in any additional ways necessary (changing filter cube, etc).
13. Prepare inner chamber insert by wrapping the agarose reservoir in parafilm. Check that agarose is melted and temperature is switched to low on heat block. Check that a clean transfer pipette is available and ready.

<u>Preparing fish for imaging (Room 157)</u>

1. Prepare a bath of Tricaine (MS-222) in system water in a clean 1L tank for initial anesthetization.
    a. for initial anesthesia (**induction**), add 9-10mL of MS-222 stock (10mg/mL) to 500mL system water.
    b. Larger fish may require a higher induction dose. Use lower concentration first, then move to higher concentration if ineffective.
2. Retrieve fish from housing. Put fish in secondary container for transport to microscope.
3. Using a clean net, transfer fish into the anesthetic bath. Wait until movement stops, but gill movement is still present. Fish should not react when soft tipped forceps are used to pinch the tail.
4. Using soft-tipped forceps, grab fish by the tail fin (avoid the peduncle/fleshy portion of the tail) and place swiftly into the prepared inner chamber insert.
5. Immediately turn chamber so that the parafilmed portion is down facing the bench, and give one or two soft taps to allow the anesthetized fish's tail to slide into the agarose reservoir.
6. If fish is positioned sufficiently with head upwards, use a transfer pipette to swiftly add a small amount of 42° C LMP agarose to the agarose reservoir while continuing to hold chamber vertically.
    a. Take care to ensure that no agarose pools around the head of the fish. There should be little to no agarose past the fish's tail.
    b. If you need to reposition the fish to keep its head pointed up, use a cat whisker tool.

7. Add a small amount of dental wax over top of the back of the fish and onto the side of the chamber to secure it in place.
8. LMP agarose should solidify quickly. If you need to, you can touch the reservoir to a coldpack to speed up the solification process.
9. Using a gloved hand, place inner imaging chamber with fish into the outer imaging chamber set up on the microscope.
    a. make sure inner chamber is fitted snugly and the fish is submerged fully in water.
    b. Check to ensure that gill movement is still present.
10. Insert intubation tubing into the fish's mouth, ensuring that the tubing only goes into the first ~1mm of the mouth.
    a. If the tubing is inserted too deeply, the fish will be gravely injured by water being forced too far into its mouth. Be aware and monitor for signs of distress or trauma.



Imaging Fish

1. Image fish according to your microscope and experimental parameters.

Monitoring the health of the animal

1. Keep track of the time that the fish remains anesthetized using the appropriate log. Fish should be revived at the 2-hour mark if they are intended to be recovered.
2. Carefully monitor for signs of laser damage to the tissue.
3. Keep laser power as low as possible.
4. Check for gill movements or signs of distress frequently.

Recovering the animal after imaging

1. Pause the peristaltic pump and switch water source to system water (no tricaine). Keep outflow tubing in original reservoir, so fresh water is flowing in and tricaine water is flowing out and not recirculating.
2. After 5-10 minutes, as gill movements begin to increase or fish begins to move, remove the fish from the chamber and place into 1L tank with system water at 28°C. Monitor for signs of distress. If the fish is active and swimming, prepare for transport back to isolation facility or other outcome in accordance with your experiment and protocol.