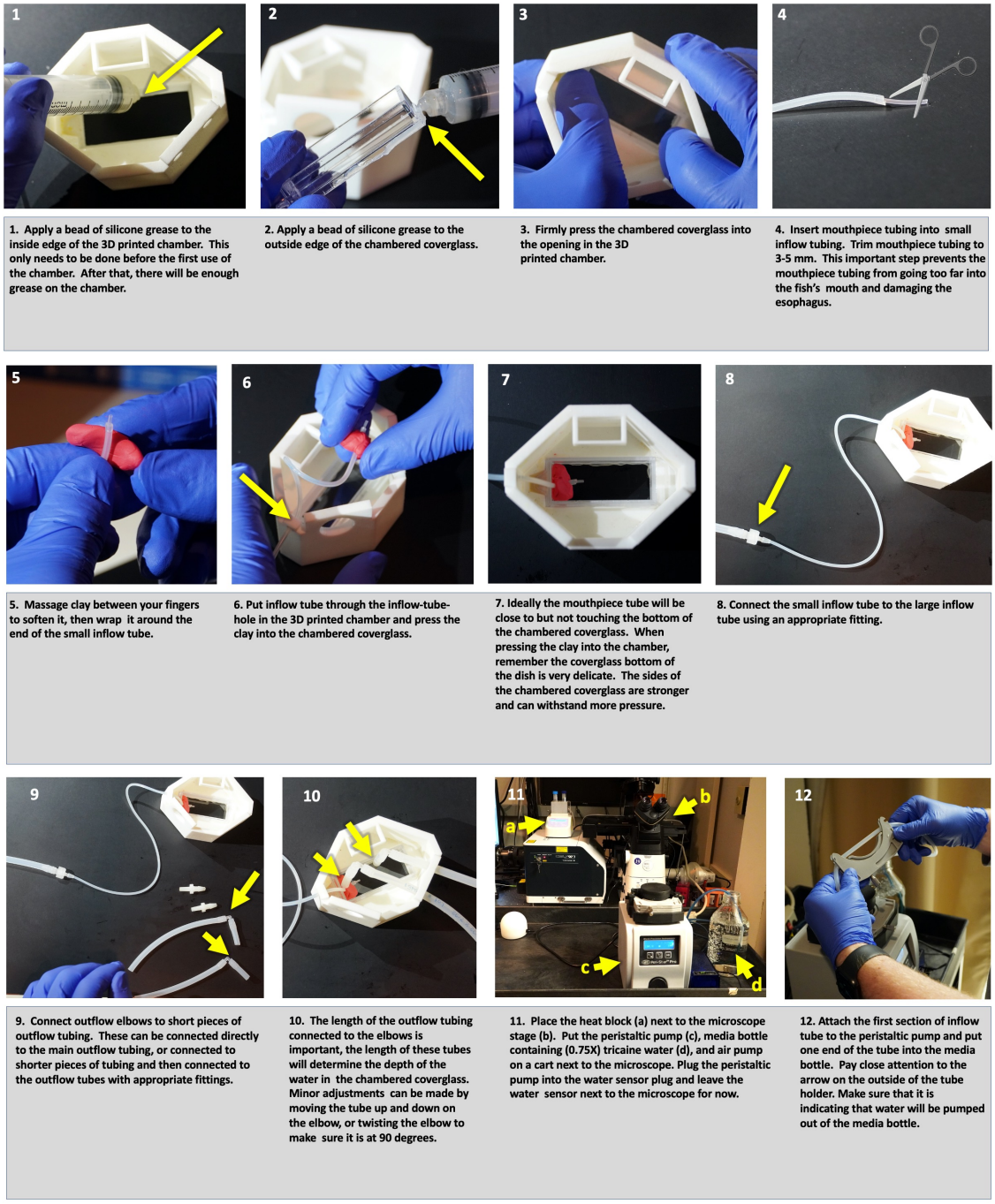
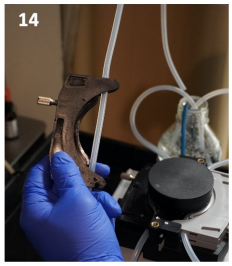


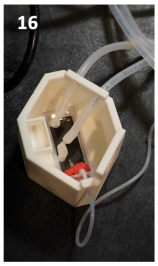
Fig S1a. Intubation Setup Instructions Part 1

13. Attach the other end of the first section of inflow tube to the pulse dampener. Attach the next section of inflow tube to the pulse dampener. This is the tube that leads to the mouthpiece tube. Make sure this tube is long enough because it will need to be wrapped several times around the heat block.

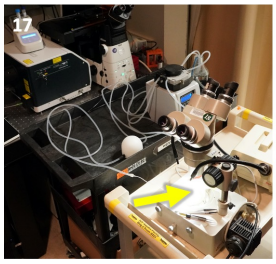


14. Attach both outflow tubes to the opposite side of the peristaltic pump as the inflow tubes and place the ends into the media bottle.

15. Turn on the peristaltic pump. It will take a few minutes for water to work its way through the tubes and for enough pressure to build in the pulse dampener.



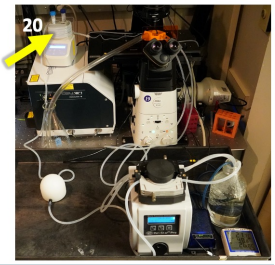
16. Let water run through the chamber for a couple of minutes then shut off the pump and prepare to mount. Pressure in the pulse dampener will continue to push water through the system for a little while. Keep an eye on the chambered coverglass to make sure that it doesn't overflow.



17. Move the imaging chamber to a nearby stereo microscope with adequate working distance to comfortably fit the chamber and allow you to manipulate tubes and the fish. If your system does not have adequate working distance, a lower magnification objective (0.5x) might be needed.

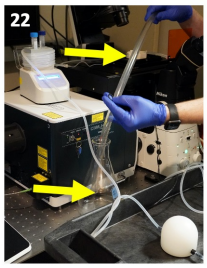
18. Anesthetize the fish you will be imaging in 1X (160 mg/L) Tricaine. Once anesthetized, transfer the fish to the chambered coverglass. Using fine forceps to manipulate the mouthpiece tube and plastic tweezers to gently maneuver the fish, holding it by the pectoral fin, use the mouthpiece tube to open the fish's mouth and gently insert the tube.

19. Moisten a sponge in tricaine water that has been cut to fit into the chambered coverglass and gently place it on top of the fish. Place both outflow tubes into the chambered coverglass and make sure they are at a level that the fish will stay covered with water but the chambered coverglass won't overflow. Turn on the peristaltic pump while the chamber is still on the stereo microscope and make sure the mouthpiece stays in the fish's mouth and the outflow tubes are working properly.



20. Place the chamber on the microscope stage. Immediately check that the mouthpiece is still in the fish's mouth and the outflow tubes didn't shift and are working properly. Coil inflow tube around the heat block. Insert overflow sensor into the chamber. Add temperature probe.

21. Apply silicone grease to the "O" ring that holds the overflow tube in place.



22. Place one end of the overflow tube in a flask or other receptacle to capture any overflow water. Insert the other end of the overlow tube into the overflow hole in the 3D printed chamber.

23. Secure the "O" ring to the overflow tube inside the 3D printed chamber.

Tips and Tricks

1. Check to make sure that you have created a watertight seal between the chambered coverglass and 3D printed chamber by filling the 3D printed chamber with water and waiting a few minutes to detect leaks. The seal should even be able to withstand gentle pressure from underneath the coverglass.

1. Periodically check the water level in the chamber. Check the outflow by making sure that the outflow tubes are dripping media into the media bottle.

2. The eggs inside of gravid females are very delicate. Too much pressure on the abdomen can damage eggs and result in fish death.

3. Fish should not be fed the day of an overnight intubation. This will prevent feces from interfering with imaging, fouling water quality, and prevent regurgitation.

4. For post-intubation revival, replace the tricaine media with non-tricaine-containing media and leave the fish intubated for approximately 15 minutes longer. After beginning the revival process, observe the fish for continuously for signs of recovery (opercular and jaw movement) using transmitted light and a low magnification objective (4X or 2X), removing the fish from the intubation chamber when these movements become pronounced and regular.

Fig S1b. Intubation Setup Instructions Part 2

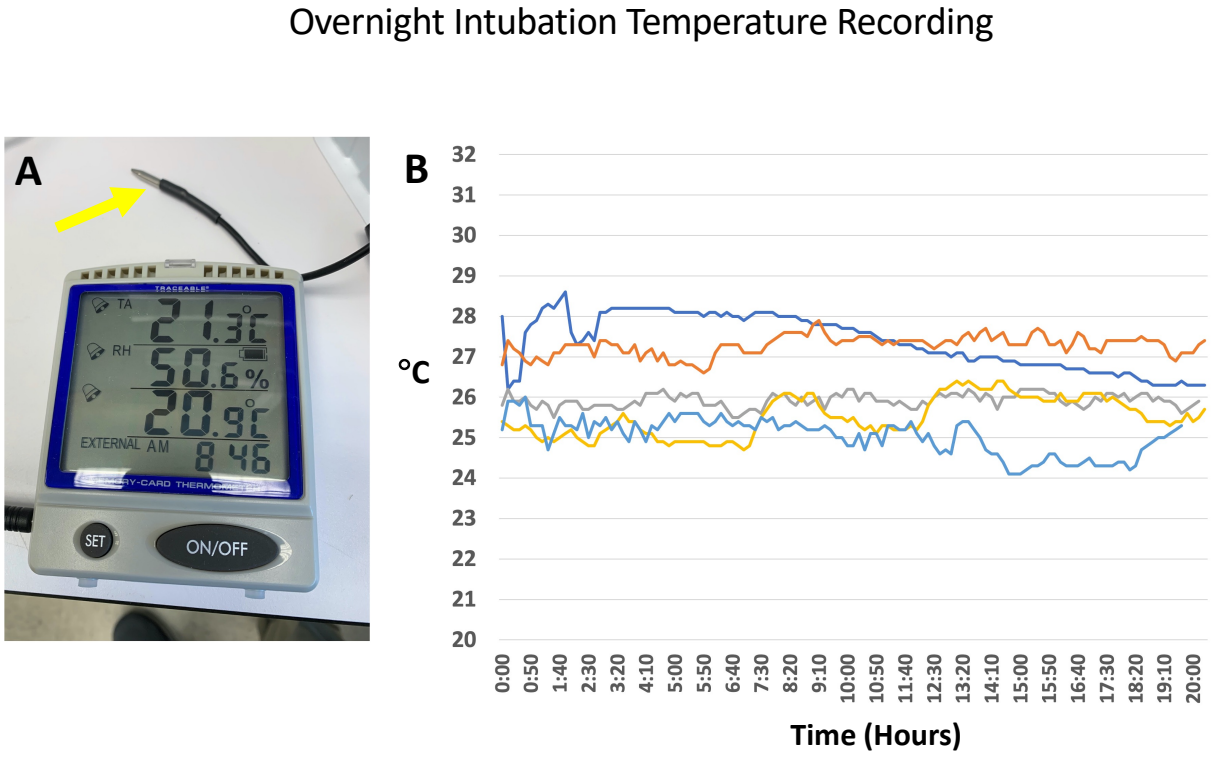


Fig. S2. Overnight Temperature Recordings of Intubation Chamber
A. Fisherbrand Traceable thermometer (cat# 15-081-111) with probe (yellow arrow) used to measure the water temperature inside the intubation chamber. **B.** Temperature plots from 5 different overnight intubations. Temperature was maintained between 24 and 28 degrees, with individual runs staying within 2 °C.

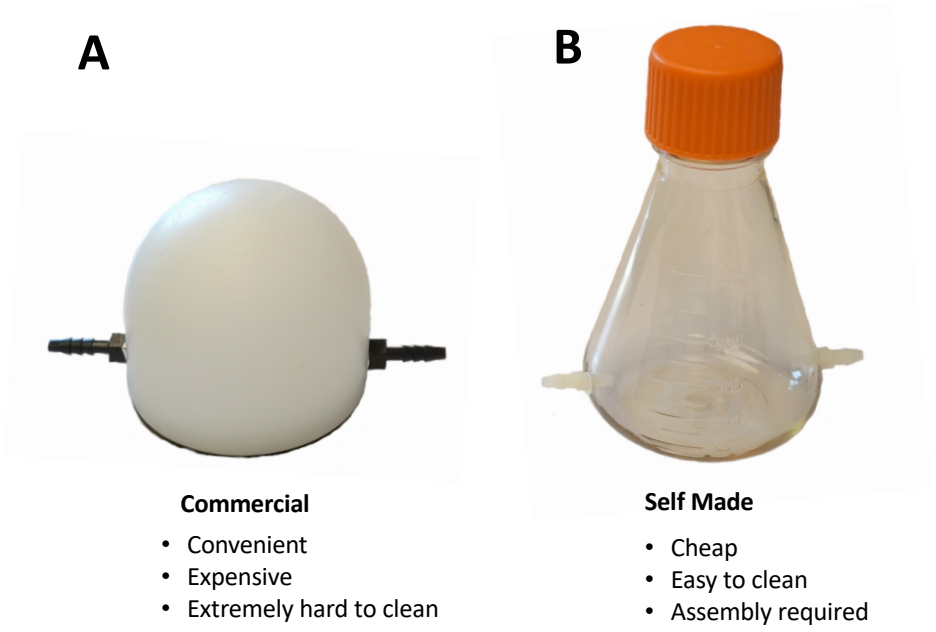


Fig. S3. Pulse dampeners are used to reduce fish movement caused by the pulsatile flow created by the peristaltic pump. A. Commercially available pulse dampener (Materflex Item# HV-07596-20). **B.** Self made pulse dampener made of a plastic Erlenmeyer flask with holes drilled in the side and tube fittings glued in with epoxy.

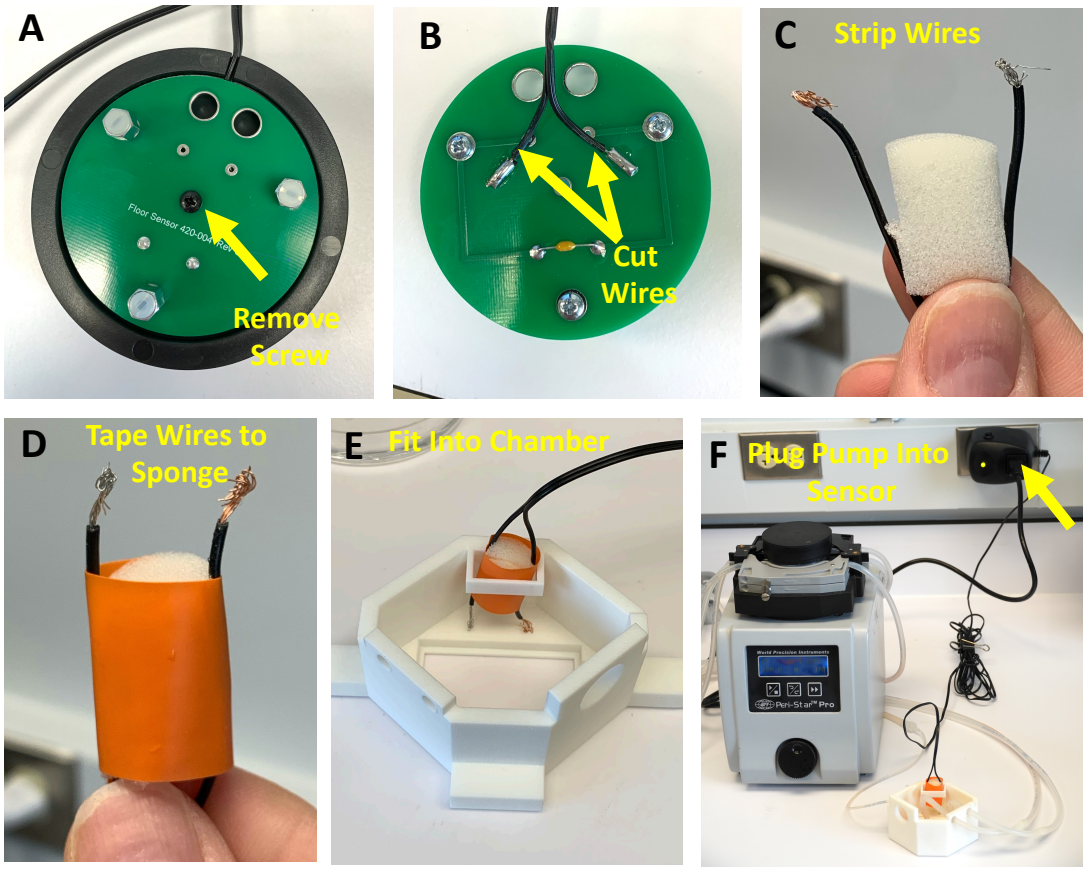


Fig. S4. Modification of water sensor to fit in 3D printed chamber
A. Ventral view of water sensor showing the screw that needs to be removed. **B.** Inside of water sensor showing where to cut wires **C.** Cut and stripped wires. **D.** The wires held around a sponge with vinyl tape. **E.** The modified sensor in the 3D printed chamber. **E.** The peristaltic pump plugged into the water sensor which is plugged into the outlet (yellow arrow).

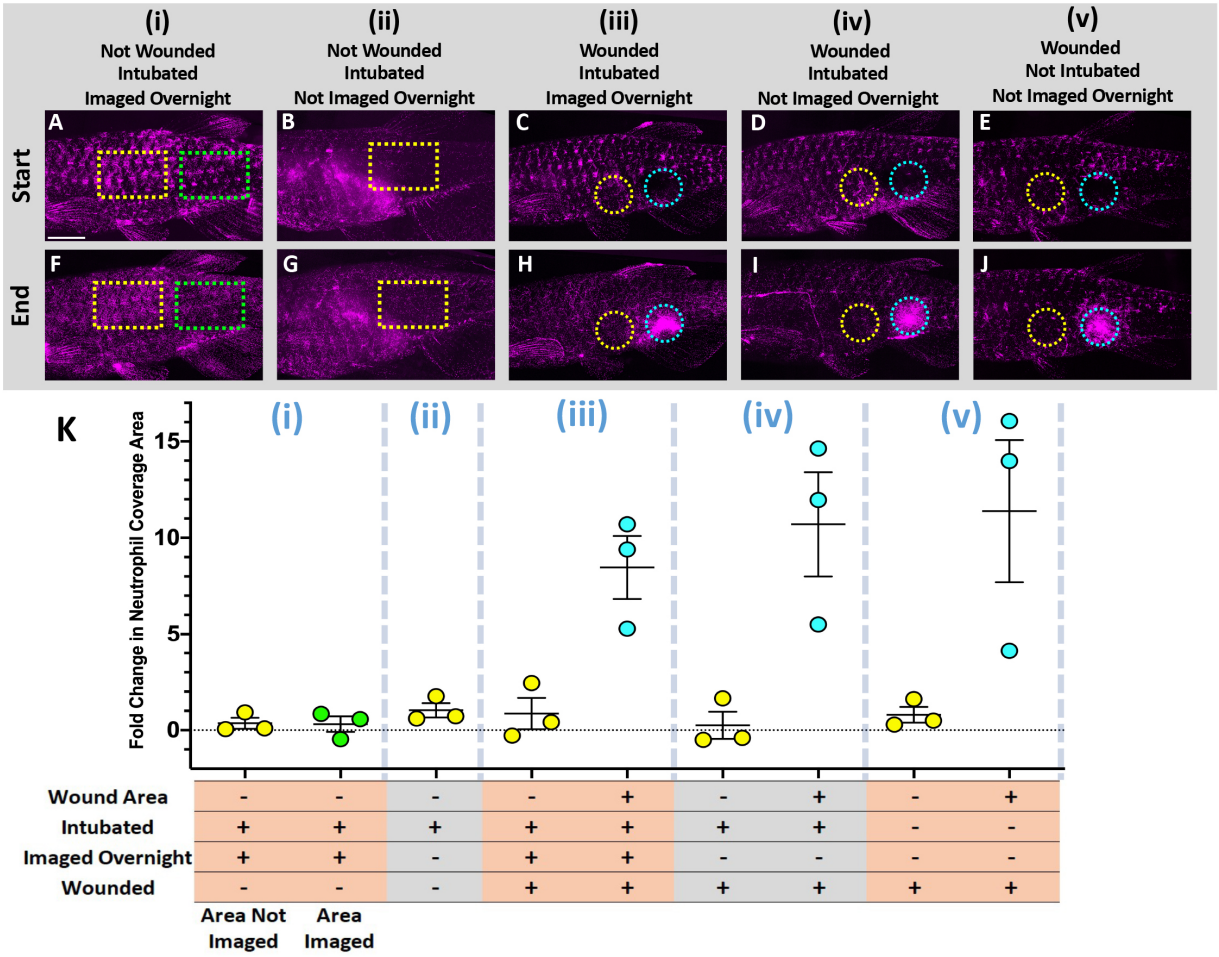


Fig S5. Quantification of changes in neutrophil coverage in adult fish in overnight experiments

A-J. Representative confocal micrographs of adult casper *Tg(lyz:DsRed2)*^{NZ50} transgenic zebrafish with fluorescent neutrophils (magenta), at the start **(A-E)** and end **(F-J)** of overnight (at least 20 hour) tests with areas quantified in panel **K** shown by dashed rectangles and circles. Five sets of 3 fish each were treated as follows: **(i)** not wounded, intubated, with the green area imaged continuously overnight and the adjacent yellow area only imaged at the start and end of the experiment; **(ii)** not wounded, intubated, only imaged at the start and end of the experiment; **(iii)** wounded, intubated, with the wounded area (cyan) and an adjacent unwounded area (yellow) both imaged continuously overnight; **(iv)** wounded, intubated, with the wounded area (cyan) and an adjacent unwounded area (yellow) both imaged only at the start and end of the experiment; **(v)** wounded, not intubated, with the wounded area (cyan) and an adjacent unwounded area (yellow) both imaged only at the start and end of the experiment; . **K**. Plot showing the mean (± SEM) fold change in neutrophil coverage area calculated for the regions shown by the rectangles and squares above. Adjacent Shaded areas (salmon and grey) represent different areas measured from the same fish (n =3 fish for each treatment). The mean area of neutrophil coverage in all three wound areas (cyan) was significantly greater than the control areas in the same fish ($p < 0.05$ Holm-Bonferroni corrected) Scale bar = 2 mm.

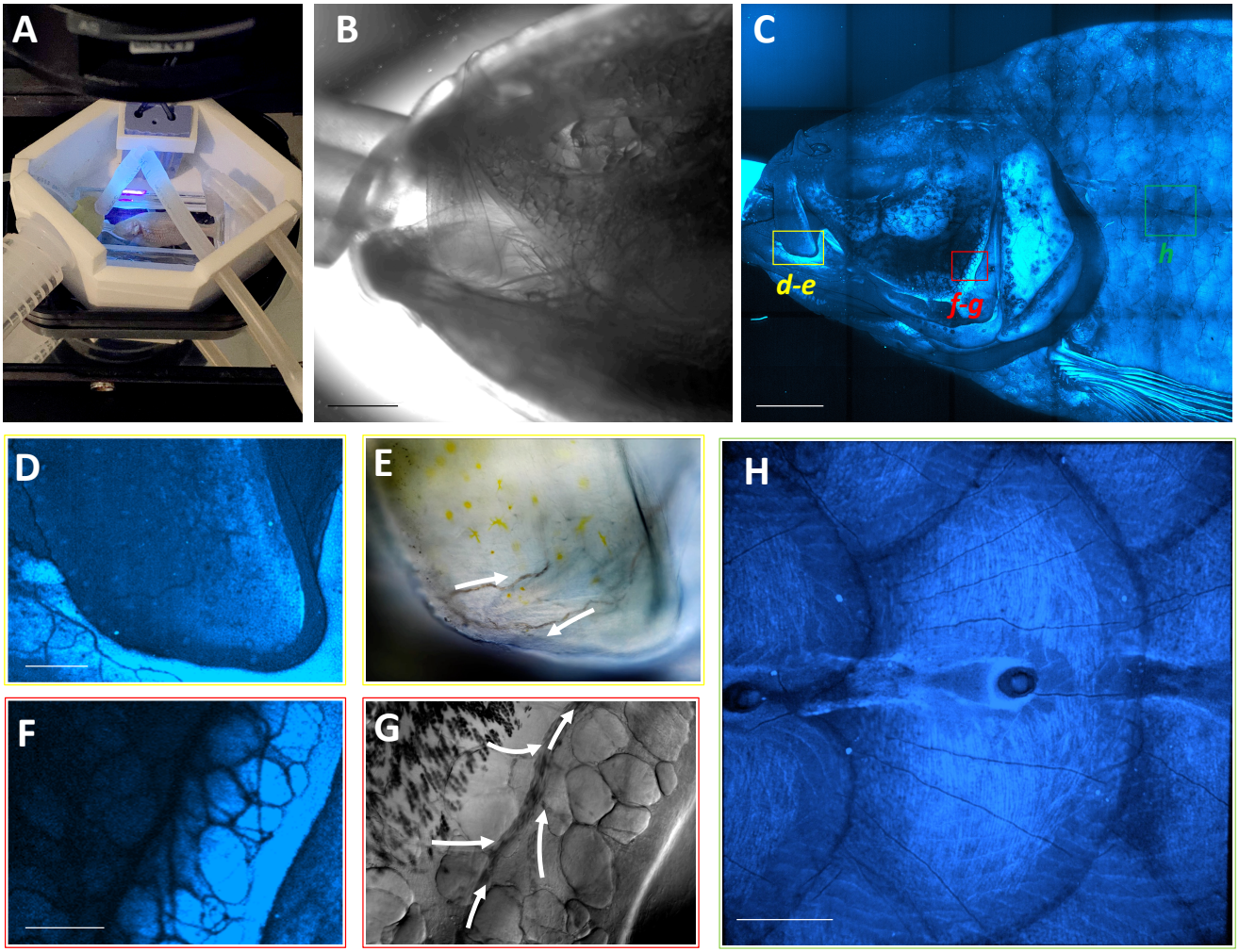
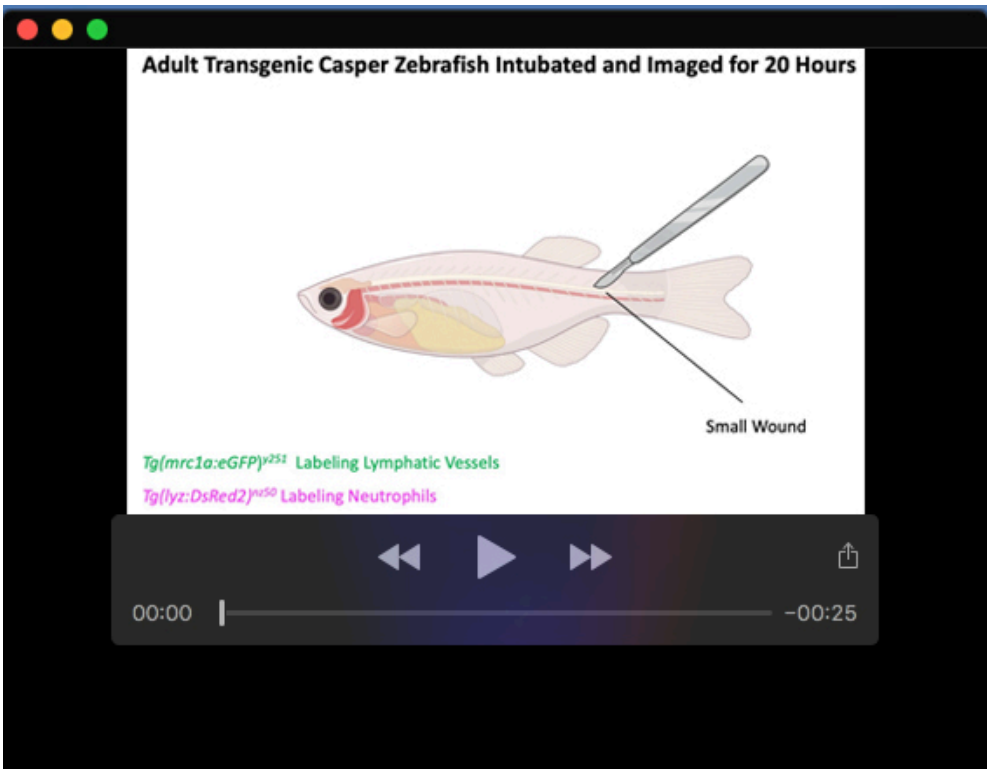


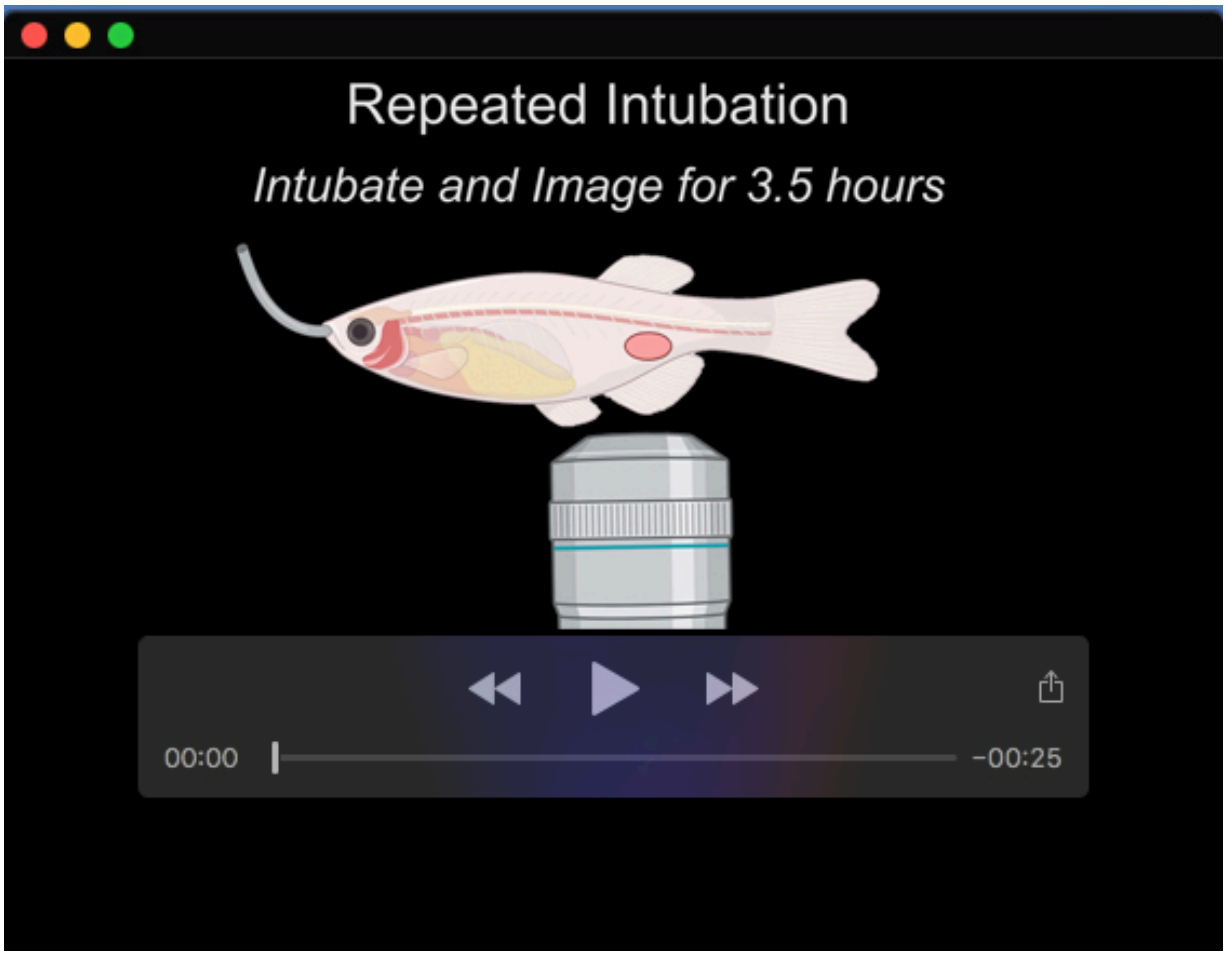
Fig. S6. Intubation and imaging of Mexican Cavefish (*Astyanax mexicanus* – Pachon)
A. Cavefish intubated and being imaged on spinning disk confocal. **B.** Close up transmitted light image of the intubation tube inside the cavefish's mouth. **C.** Overview confocal tile scan of autofluorescence in the bones and scales of the cavefish. **D-E** Close up fluorescent (**D**) and transmitted light (**E**) image of the maxilla with white arrows showing the direction of blood flow. **F-G** Close up fluorescent (**F**) and transmitted light (**G**) image of the preoperculum with white arrows showing the direction of blood flow. **H.** Close up of autofluorescence in a scale showing the lateral line. See **Supp Movie 3** for videos. Scale bars: **B** 1 mm, **C** 2 mm, **D,H** 250 μm, **F** 200 μm.

Table S1. Materials List

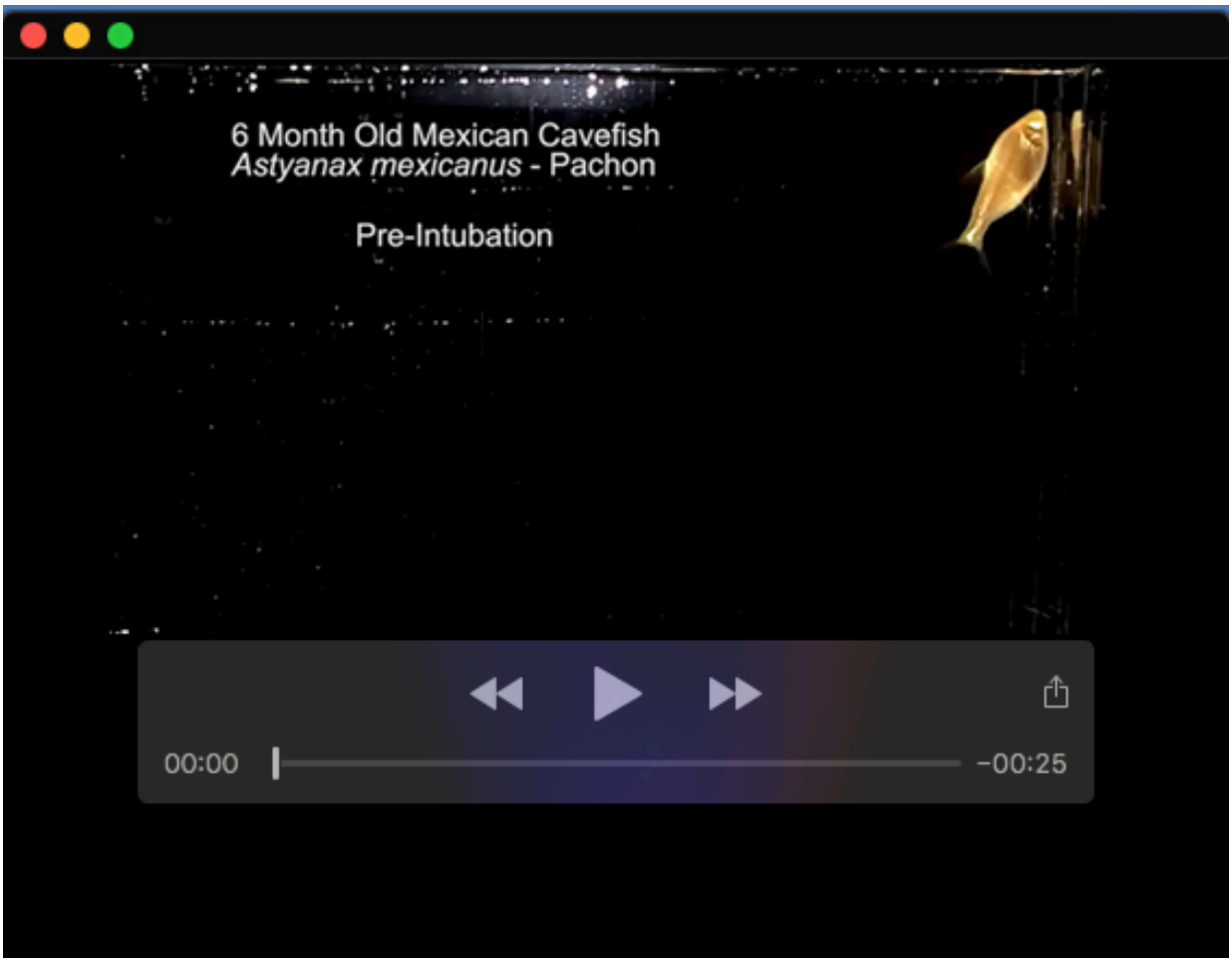
Item	Description	Supplier	Part Number
3D printed Chamber	Fits on microscope stage, holds LabTek dish	Xometry / Xometry.com	3D renderings in supplement, Nylon 12 (SLS)
Lab Tek II Imaging Dish	Single chamber glass bottomed imaging dish #1.5 coverglass	ThermoFisher / ThermoFisher.com	Lab-TekII #155360
Peristaltic Pump	Pumps fish water through chamber	World Precision Instruments / wpiinc.com	World Precision Instruments Peri-Star™ Pro Cat# PERIPRO -4L
Silicone Grease	Seals Lab Tek dish into chamber	Grainger / grainger.com	SG-ONE™ 24708
Water Supply Tubing (pump)	Supplies water to chamber through peristaltic pump	US Plastics / usplastic.com	Tygon 3350 3/32" ID X 7/32" OD
Small Inflow Tubing	Brings water from supply tubing into chamber	US Plastics / usplastic.com	Tygon 3350 1/32" ID X 3/32" OD
Intubation Tubing	Small tube that fits in fish's mouth	World Precision Instruments / wpiinc.com	Flexible PE Tubing, 0.6mm ID, 1.1mm OD # 504280
Overflow Tubing	Large diameter tubing for emergency overflow	US Plastics / usplastic.com	Tygon S3 E-3603 ½" ID, ¾ OD, 1/8" wall
Outflow Elbows	Elbow that changes the direction of the outflow tube inside the 3D printed chamber	US Plastics / usplastic.com	1/8" to 5/32" Kartell Polypropylene Elbow Connector # 64256
Overflow Fitting	Attaches overflow tubing to chamber	US Plastics / usplastic.com	1/2" to 5/8" Kartell Polypropylene Straight Hose Barb Connector #64114 (*one end removed*)
Overflow Gasket	Gasket that holds overflow fitting in place	Grainger / grainger.com	Part of O-Ring Kit ASTM D2000
Tube Fitting Kit	Attaches tubes together	World Precision Instruments / wpiinc.com	Luer-to-Tubing Coupler Assortment Kit # 504954
Modeling Clay	Holds intubation tube in place	Various	Dixon Modeling Clay DIX00740
Emergency Shut Off Switch	Modified "WasherWatcher Laundry Tub Overflow Protector" or "WaterWatcher Leak Detector Alarm"	HydroCheck, STAK Enterprises Inc. www.hydrocheckproducts.com	WasherWatcher Leak Detector Alarm
Sponge	Holds fish in place	Cole Parmer / Coleparmer.com	Jaece Identi-Plugs L800-D
Heat Block	Warms fish water	Southwest Science / southwestscience.com	SH100 Mini Dry Bath Hot Block, SWMINI-5 block insert
Digital Thermometer	Thermometer for monitoring and recording chamber water temperature	Fisher Scientific / Fishersci.com	Fisherbrand Traceable Thermometer Cat# 15-081-11
Air Pump With Air Stone	Aerates fish water in water bath	Various	Any, for small home aquarium
Tokei Hit Heated stage A	Keeps microscope samples warm	Tokei Hit / Nikon Instruments	Model: INUB-TIZB
Tokei Hit Heated stage B	Keeps microscope samples warm (fits 96 well plate)	Tokei Hit / Nikon Instruments	Model: STZF-TIZWX-SET
Soft Tip Tweezers	Tweezers used to manipulate fish for intubation	Fisher Scientific / Fishersci.com	Exceltra 162DRT Cat# 18-100-921
Pulse Dampener	Reduces pulsatile movement caused by pump	Masterflex / Masterflex.com	Masterflex Item# ZY-07596-20



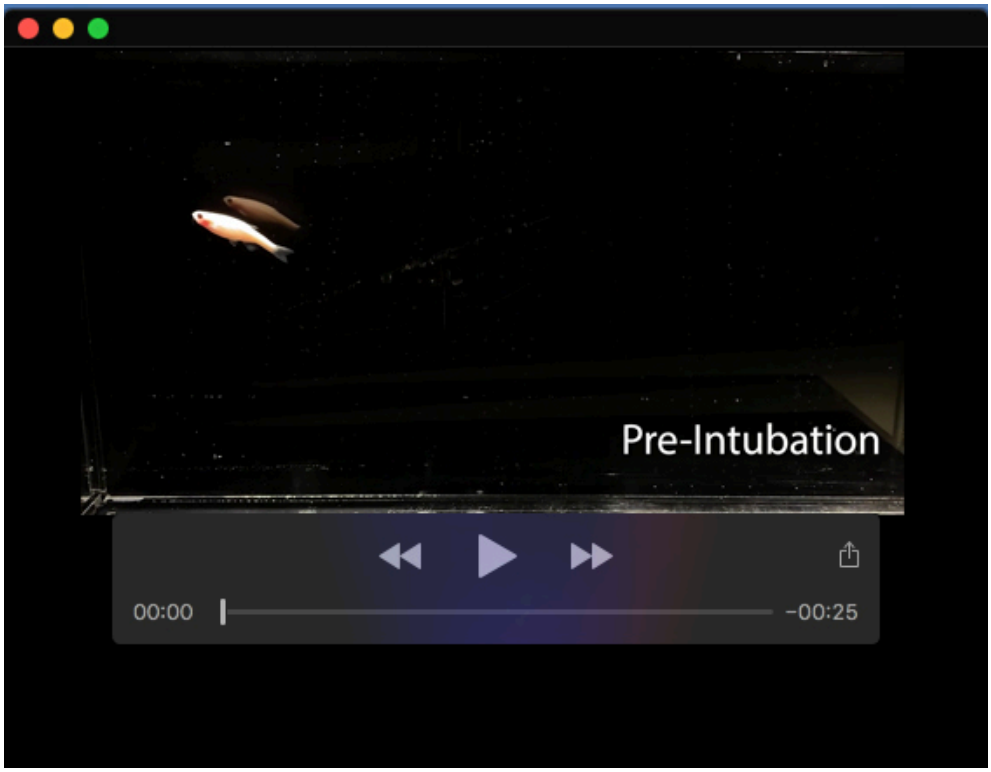
Movie 1. Time-lapse imaging of neutrophil recruitment to a scale removal wound in an intubated adult zebrafish. 0-4'' Schematic diagram an adult casper *Tg(lyz:DsRed2)*^{NZ50}*;Tg(mrc1a:eGFP)*^{y251} double transgenic zebrafish with fluorescent neutrophils (magenta) and lymphatic vessels (green). The approximate site of scale removal by abrasion with a scalpel is noted with a yellow box. **5-10"** Adult fish being imaged in the intubation chamber on an inverted confocal microscope, using blue, green and purple (near-UV) excitation light. **11-31**" Time-lapse imaging of the wound site of an adult fish collected with a 2X objective from 0 – 19 hours post wounding and intubation **31-38"** Close-up of the wound site collected with a 10X objective at 19-20 hours post wounding and intubation. **38-44"** High magnification closeup of neutrophils (magenta) actively migrating in and around lymphatic vessels (green) in the recovering wound site of a live adult zebrafish collected with 20X objective from 23 – 24 hours post wounding and intubation.



Movie 2. Repeated time-lapse imaging of neutrophil recruitment to a scale removal wound in an intubated adult zebrafish. **0-14"** Schematic diagram showing experimental setup: an adult casper *Tg(lyz:DsRed2)*^{NZ50}*;Tg(mrc1a:eGFP)*^{y251} double transgenic zebrafish with fluorescent neutrophils (magenta) and lymphatic vessels (green) is wounded, intubated and imaged for 3.5 hours then returned to the aquaculture system. Imaging and intubation (3.5 hours) is repeated at day 1, 2, and 7 post injury. **15-25"** Time lapse, 0.5-4 hours post injury. **25-34"** Time lapse, 24-27 hours post injury. **35-42"** Time lapse, 48-51.5 hours post injury. **43-52"** Tile image of entire intubated fish at 7 days post injury, zooming to wound site. **53-1:09"** 3.5 hour time lapse of wound site at 7 days post fertilization including increased magnification.



Movie 3. Intubation and imaging of Mexican Cavefish (*Astyanax mexicanus* – Pachon). 0-6” A six-month old cavefish swimming before intubation. **7-12”** Cavefish intubated and being imaged on an inverted spinning disk confocal microscope. **13-17”** Overview of 405 nm autofluorescence tile zooming in to scale/lateral line region. **18-27”** 3D rendering of scale lateral line region. **28-33”** Pan from scale to preoperculum. **34-41”** Transmitted light movie (black and white) in real-time showing blood flowing through the preoperculum. **42-49”** Pan from preoperculum to maxilla. **50-57”** Transmitted light movie (color) in real-time showing blood flowing through maxilla. **58-1:02** Pan from maxilla to entire mouth showing intubation. **1:03-1:23”** Transmitted light movie (sped up 15X) showing cavefish being revived with fresh system water. **1:24-1:35**” Cavefish swimming after 3.5 hour intubation.



Movie 4. Adult zebrafish pre intubation, being intubated, being revived, and post intubation. 0-10”Adult zebrafish swimming before overnight intubation. **10-28”** Intubation tube being carefully inserted into the mouth of an adult zebrafish. **29-57”** Intubated adult zebrafish being revived by substituting fresh system water for tricaine water in the intubation system. **58-1:06”**Adult zebrafish swimming after overnight intubation.