Doris Wu, 2009, revised

Paint-fill technique

Equipment

1) Manual manipulator right hand M3301R and a broad steel base for mounting manipulator, World Prcision Instruments Tel. 941-371-1003
2) Hamilton 80601 710LT syringe100ul
3) 19 gauge needle
4) Glass capillary tubing. Borosil 1.0x0.75 mm ID/fiber catalog # 30-30-0 from FHC Tel:207-666-8190
5) pipet puller
6) methylsalicylate, glycerol
7) 60mm glass petri dish with elastomer (follow instruction in Kit, add charcoal to darken the elastomer)
8) SV11 dissecting microscope with fiber optics
9) Clay Adams, Division of Becton Dickinson, New Jersey #7420 Intramedic polyethylene tubing ID .86mm.
10) 184 silicone elastomer kit from Dow Corning Corporation, Midland Mich. World Precision Science
11) Exterior Alkyd gloss white paint or White out fluid. The paint or White out is not soluble and is a suspension. If solution is clogging your injection needle, quick spin to remove the larger particles and use the supernatant.

For 100 ml Bodian fix:
5ml glacial acetic acid
5ml formaldehyde (straight from the bottle, approx. 37% formaldehyde)
15 ml water
75 ml 100% ethanol

Tissue preparation:

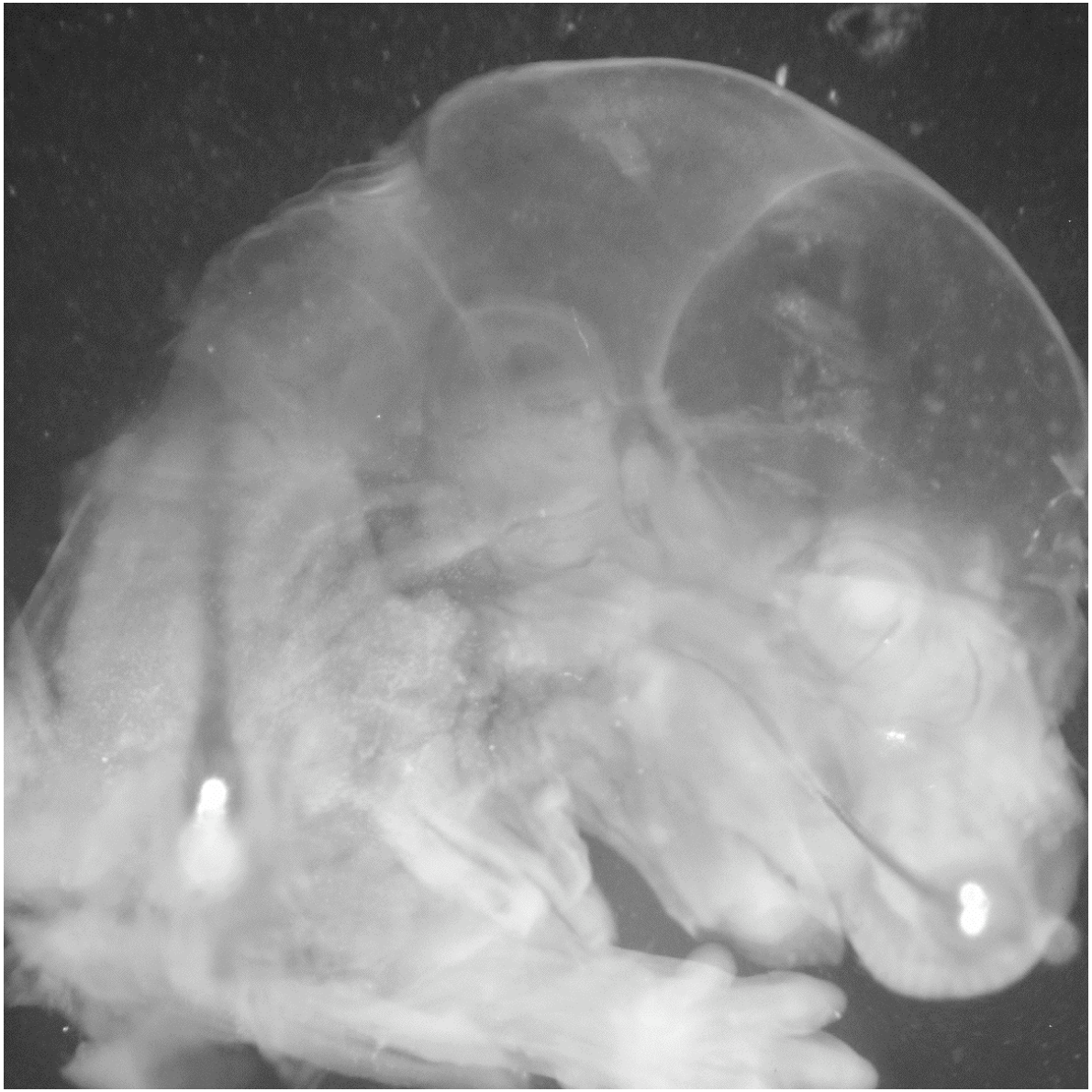
1. Fix embryos in Bodian solution overnight in 20 ml scintillation vials. For embryos older than E14.5, remove bodies below the level of forelimbs before fixation. For postnatal animals (P1-P3), hemi-section the heads in addition before throwing them into fixative.
2. The next day, wash specimens with 100% ethanol once and leave in ethanol overnight.
3. The next morning, rinse once with methyl salicylate and leave specimens in methyl salicylate until the soft tissues are cleared.

Injection:

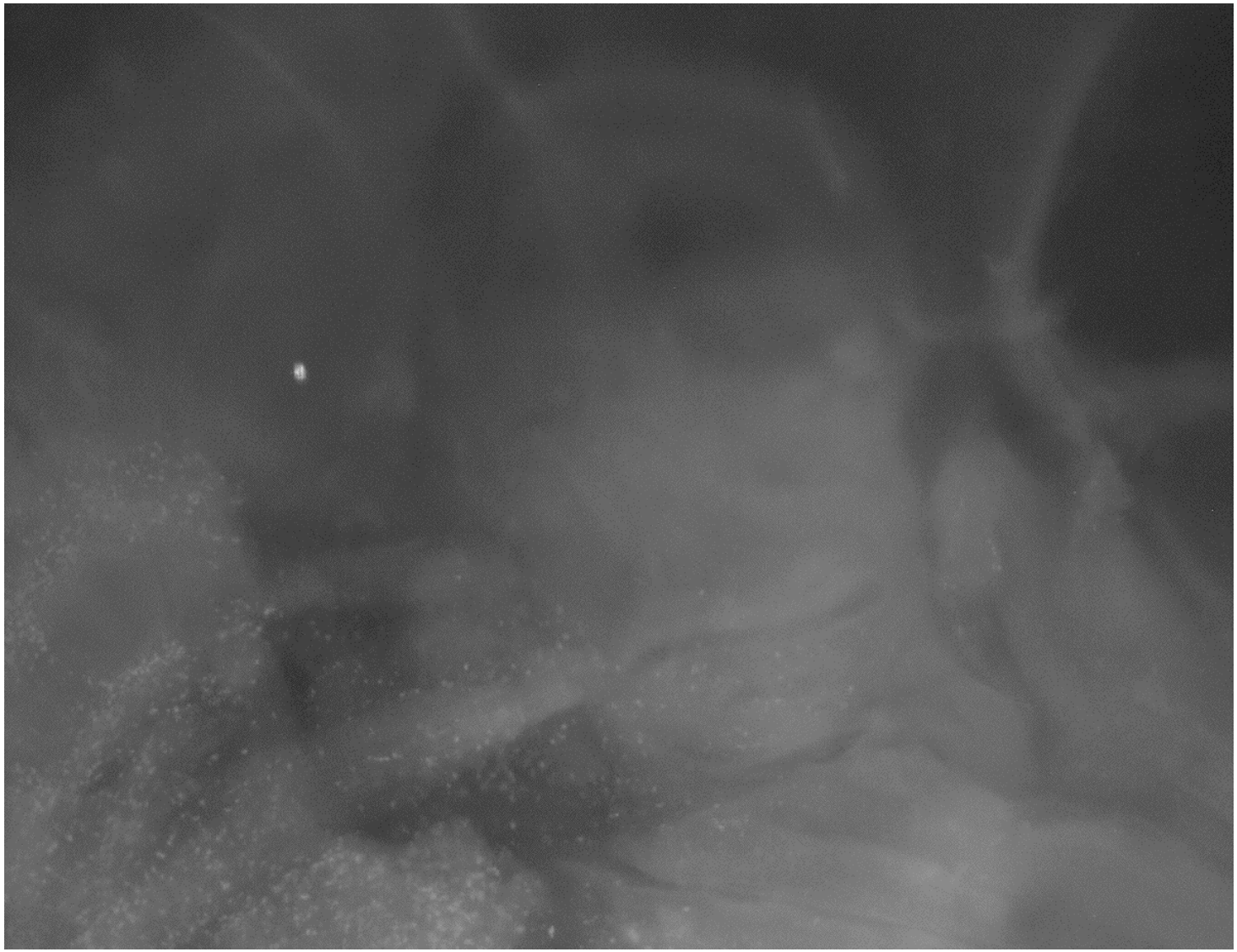
1) Cut off half of a 19 gauge needle and attach a small piece of polyethylene tubing (~ 3cm) to the cut end.
2) Attach the needle to a Hamilton syringe.
3) Fill syringe with glycerol and mount the syringe onto the micromanipulator.
4) Remove air bubbles from the system.
5) Pull a glass capillary tubing, back fill with newly vortex diluted paint or White out fluid in methyl salicylate (ranges between 0.025 to 0.1%). The amount of paint to use is empirical and is dependent on how white you want to the specimen to be. Attach capillary needle to the polyethylene tubing on the Hamilton syringe.

6) The specimen is placed under the microscope in a Petri dish with black elastomer filled with methyl salicylate. The cavity of the inner ear is visualized and injected (see ppt slides for orientation). For embryos up to E13.5, injection is approached laterally. Embryos older than E13.5 are hemisectioned and injections are approached medially through the cochlear duct or utricle area.
7) Depending on the size of specimens, the glass capillary tubing is cut accordingly right before injection.

Hemisection the mouse head
Do you see the ear?

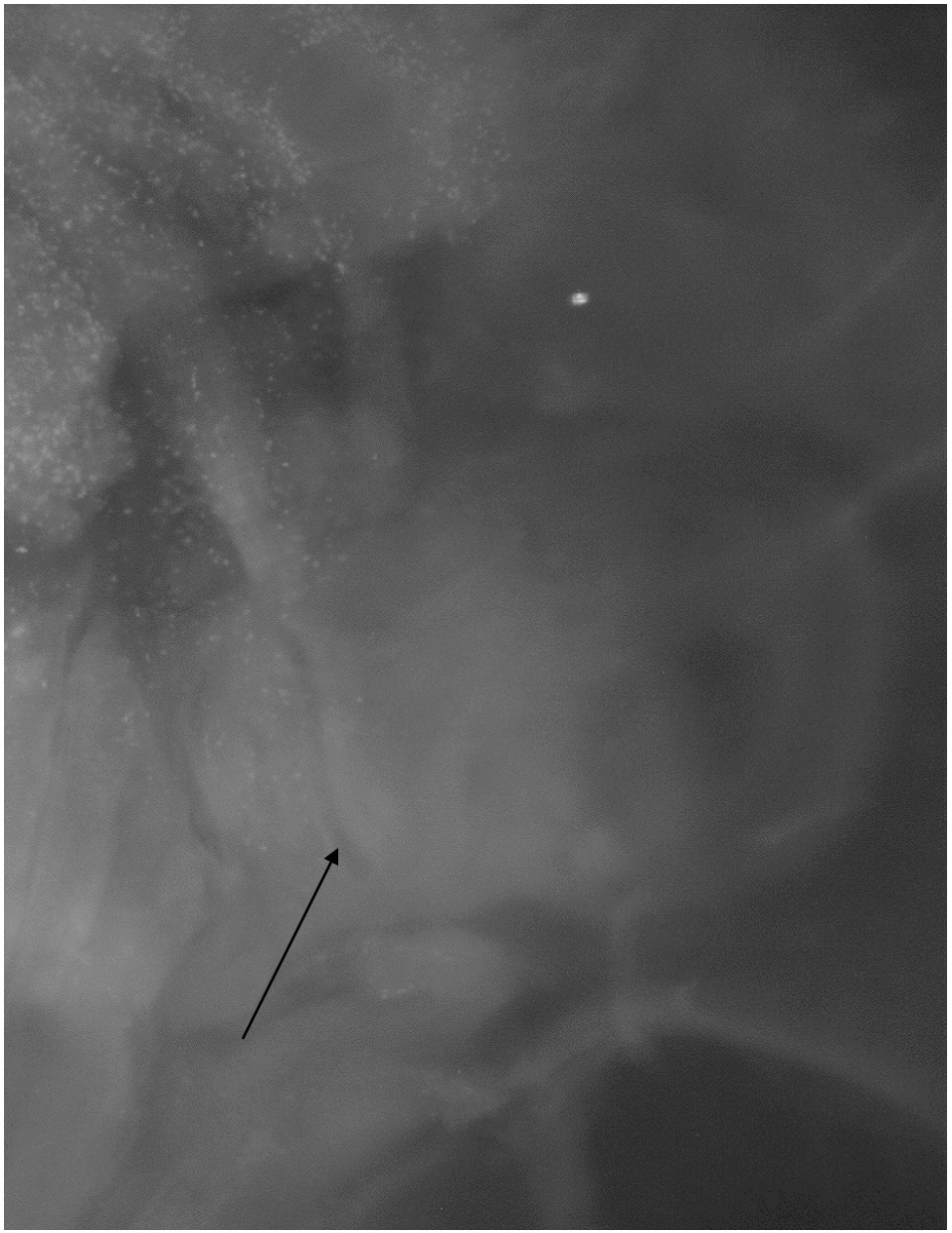


Magnified view of the inner ear

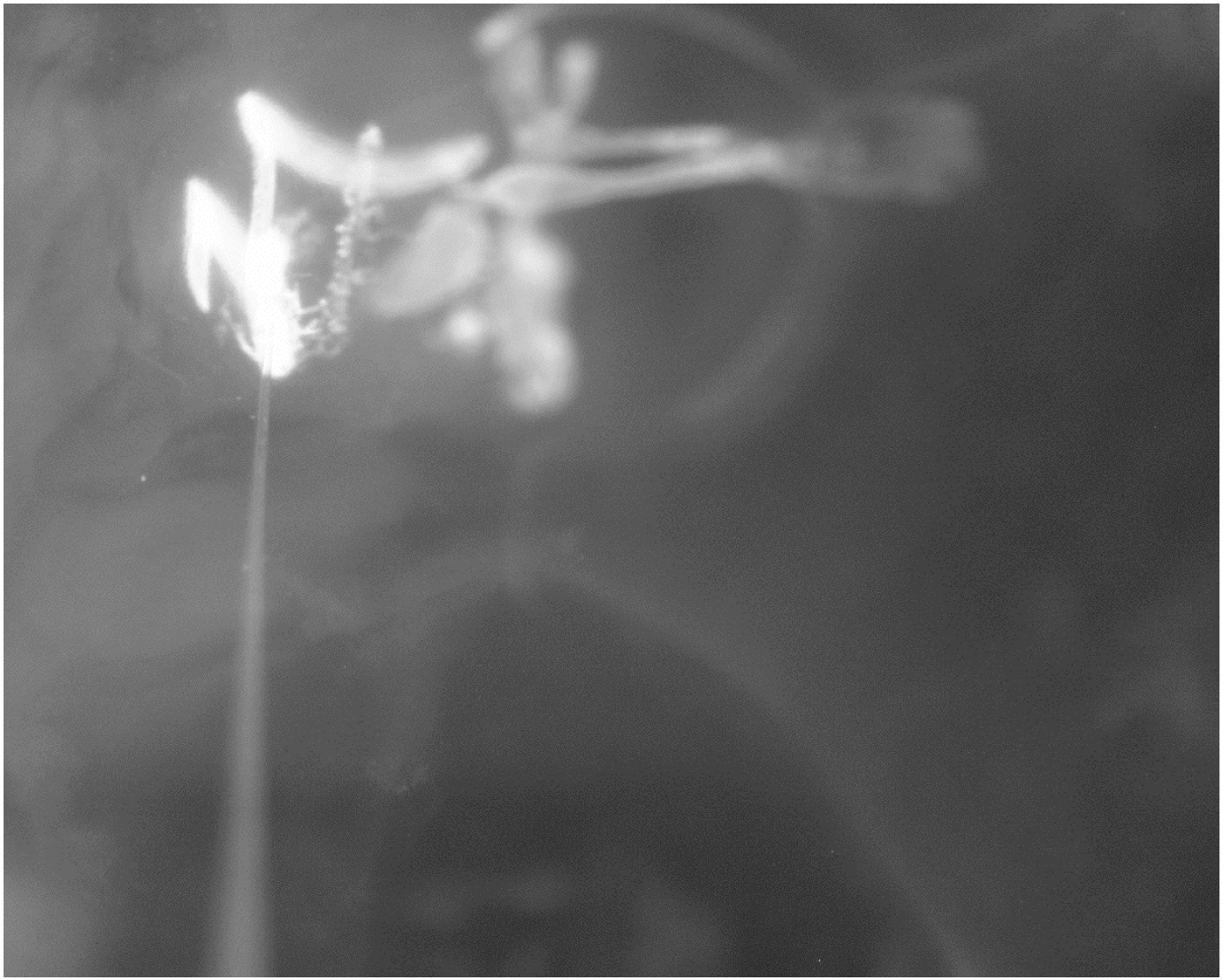


Rotate specimen 90 degree clockwise from previous orientation

Aim at the cochlear region with the arrow



Injected specimen



For utricle injection, turn specimen 180 degree from the orientation in Slide 1. Aim for area by the arrow.