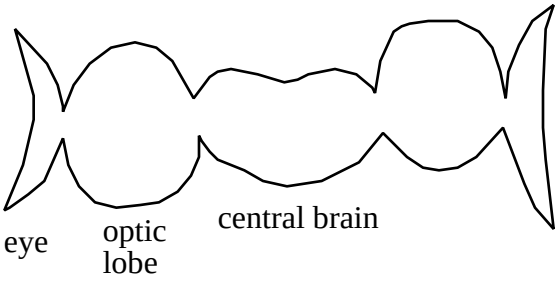
Cobalt Sulfide staining of pupal eyes

1. Collect white prepupae. Age 55 hr. at 25˚C.

2. Remove aged pupae from the side of the vial using a wet paintbrush. Blot dry on a kimwipe. Place pupa on a piece of 2-sided tape on a Sylgard dish. Using watchmaker's forceps, carefully peel away the pupal case. Don't worry too much about stabbing the pupa at this stage.

3. Place the pupa into a drop of PBS (it will float). With the ventral side up, hold the pupa by the legs with one forcep and grab the proboscis with the other. Pulling up and back, peel open the head (all the contents will explode out). Finding the eye/brain complex is the hardest part- I grab the head cuticle and shake gently. Usually all of the fat and other stuff will shake off. You are looking for something that looks like this:



   Sometime the optic lobe/eye falls off the rest of the complex. If the eye is sticking to the overlying cuticle, then the animal is too old.

4. Fix for 5 min. in 2% glutaraldehyde in PBS. Wash for 5 min. in PBS.

5. Transfer to a solution of 2% $Co(II)NO_3$ in H_2O for 5 min.

6. Using a fine tungsten needle (this is important!!!) stab the central brain or optic lobe to pick up the complex and place in a drop of water to wash briefly. Wash by swirling slowly in water three times. It is important to use a tungsten needle at this stage because you don't want to transfer too much $Co(II)NO_3$ solution to the ammonium sulfide, or you will precipitate a huge cloud of black stuff that will obscure the tissue.

7. Plunge the complex into a drop of 1% ammonium sulfide (made fresh in water) for ~30 sec. The entire tissue will appear to turn dark black- this is okay. Remove the tissue to water while you stain the rest of the your samples.

8. Mount in Aquamount (Baxter) or 70% glycerol by dissecting off the eye. Try to remove as much of the lamina (the connection between the eye and the optic lobe) as possilbe, and mount apical (outer) side up.

That's it!!!