

## COBALT STAINING OF PUPAL EYE DISCS

N.B. Make 1% ammonium sulfide (in H<sub>2</sub>O) fresh each time.

- 1) fix discs 5 minutes in 2% glutaraldehyde/PBS
- 2) wash 30 seconds in water
- 3) put discs in 2% Co(NO<sub>3</sub>)<sub>2</sub> /H<sub>2</sub>O for 5 minutes (while waiting, put down a fresh dot of diluted ammonium sulfide)
- 4) swirl discs 4 times in water
- 5) put discs in drop of ammonium sulfide ~10 seconds
- 6) wash in water
- 7) mount (we use gelmount -- it's water-soluble)

The quality of the stain is a little inconsistent; you may have to do it a few times to get a really good-looking disc to score. Also, beware that (in gelmount, at least) the cobalt diffuses away pretty rapidly; you only have about a day to evaluate your results. We take lots of pictures and score from prints.