

Fixation for larva and pupa

- 1) dissect in PBS
- 2) fix in PLP for 30'
- 3) Wash 3X10' in PBS
- 4) fix 30' in 2% glutaraldehyde (from freezer) in PBS
- 5) wash 3X 10' in PBS
- 5) post-fix in $\text{OsO}_4/\text{K}_3\text{Fe}(\text{CN})_6$ for 60'

add equal volumes of OsO_4 and $\text{K}_3\text{Fe}(\text{CN})_6$ from tubes in freezer container marked "Microscopy".

- 6) Wash 3X10' in PBS
- 7) Dehydrate in EtOH series: 30%, 50%, 70%, 80%, 95%, and 3X100%

!!!!!!!!!!No cheating!!!!!!!!!!!!!! This is critical for EM!!!!!!!!!!

- 8) 3X10' in propylene oxide
- 9) overnight in 1:1 propylene oxide:EM resin

use gloves

- 10) 5 hrs in 100% resin; imbed