**Preparation**

**\*Use fume hood at all steps possible for dyes\***

**Safranin O Solution (1%)**

1. Make 200mL of 50% EtOH solution using 105mL of 95%EtOH and 95mL of diH2O.
2. Measure out 75mL of the solution into a brown glass bottle and another 75mL into one of the staining dishes (for future use).
3. Measure out 0.75g of Safranin O and add to the brown bottle with the EtOH solution.
4. Mix, store in RT, dry, well ventilated place away from the light.
5. Do this a second time for a total of 400mL of Safranin O solution

**70% Ethanol Solution**

**\*use 95% ethanol\***

**\*you can either make stock solution beforehand, or make it as you go**

1. Make 200mL of 70% EtOH solution using 147mL 95% EtOH and 53mL of diH2O.

**50% Ethanol Solution**

**\*use 95% ethanol\***

1. Make 200mL of 50% EtOH solution using 105mL of 95%EtOH and 95mL of diH2O.

**Procedure for Safranin O (20-30 slides at a time)**

**\*Do all steps in fume hood\***

1. Label jars (Safeclear II, 1:1 Safeclear II and EtOH, 100% EtOH, 95% EtOH, 70% EtOH, and 50% EtOH). Arrange jars in this order in a line.
2. Put about 300mL of the designated solution in each jar (measure out 300mL of the first jar, then fill the rest to about the same line). Put lids on jars after they have been filled (for the last jar, put paper towel over it).
3. Put slides in slide rack. Submerge slide rack into the jar labeled “Safeclear II” and let sit for 5 minutes.
4. Take slide rack out and place in the jar labeled “1:1 Safeclear II and EtOH,” let sit for 5 minutes.
5. Remove slide rack and place in the jar laveled “100% EtOH” and let sit for 5 minutes.
6. Leave slide rack in the jar labeled “100% EtOH” and dump out the ethanol into the sink. Pour in fresh 100% EtOH until it is level with the other jars. Let sit for 5 minutes.
7. Transfer the slide rack down the line of solutions, leaving it in each solution for 5 minutes.
8. While doing the various EtOH washes, dump out the “Safeclear II” jar. Rinse with a bit of ethanol and wipe it out. Pour the Safranin O solution into the jar and cover with a lid. Put jar at the end of the line.
9. After the slides have been in the 50% EtOH solution, transfer them to the Safranin O jar. For thick leaves (green/some orange slides) leave slides in the stain for about 2 minutes (no longer). For thin and small leaves (pink/some orange slides) leave in stain for only 30 seconds to 1 minute.
10. After the slides have stained, transfer them to the 50% solution jar. Let them rinse for only 30 seconds with agitation.
11. Transfer slides to 70% EtOH solution and let sit for 1 minute. If still too dark, leave them in for an extra minute.
12. Transfer slides to the 95% EtOH jar and let sit for 1 minute.
13. Transfer the slides to the 100% EtOH jar and let sit for 1 minute. If still too dark, dump out the ethanol and add fresh 100% EtOH and let sit for another minute.
14. Clear the slides
    1. If using clove oil to clear:
       1. Use a dropper to drop some oil on each slide to remove remaining alcohol (dispose of this run-off)
       2. Then dip slides into staining jar filled with clove oil for about 10 seconds
       3. Remove slides and transfer them to a jar of Safeclear II for 1 minute
       4. Mount
    2. If using Safeclear II:
       1. Place slides in jar with 1:1 Safeclear II and 100% alcohol for 5 minutes
       2. Transfer to pure Safeclear II for 5 minutes
       3. mount