

### Experiment No. 1:

**Aim:** Demonstration of double staining technique and Preparation of double staining slides

#### Material required:

1. Sharp razor,
2. Brush,
3. Dropper,
4. Needles,
5. Watch glass,
6. Microscopic slides,
7. Cover-slips,
8. Safranin,
9. Methyl green
10. Glycerine and
11. Compound microscope.

Double stain – Safranin-O & Methyl green				
Stain	Colour of stained structure	Molecule stained	Structure stained	Other features
Safranin-O	red	lignin	Xylem	thick
Methyl green	Blue, blue-green	cellulose	Phloem, pith,epidermis	thin

**Theory:** Staining method 1- double stain – safranin-O and methyl green Cell walls composed mostly of cellulose will appear thin and blue or blue-green (stained with methyl green) while those containing lignin will appear thicker and red-orange or red (stained with safranin-O)

#### Technique:



(Steps of staining process)

1. Take 2-3cm long pieces of the material.
2. Hold the material between thumb and first finger of your left hand.
3. Hold the razor in the right hand with edge of the blade facing you and handle at right angle to it.

4. Dip the top of the material in water.
5. Then start cutting transverse sections as fast as possible in a watch glass containing water.
6. Select the thinnest section of the material with the help of a delicate brush.
7. Take a clean watch glass with water, transfer thin sections of the material.
8. Using forceps, gently transfer the sections into a small petri dish or well (separated them from the wax as you do this) and immediately add a drop of safranin stain to cover the sample. Let the section sit in the stain for 3 minutes.
9. Remove safranin by blotting it with filter paper and immediately add 70 % ethanol. Let the section sit in 70% ethanol for 1 minutes. Remove the ethanol wash, add more 70% ethanol for another 1-2 minutes to completely remove excess safranin from the section.
10. Remove the remaining 70% ethanol by blotting and immediately add methyl green and let it sit for 1 minute. Remove methyl green by blotting.
11. Immediately add 95% ethanol and allow it to sit with gentle agitation for 1 minute. Remove the ethanol wash. Add more 95% ethanol for another 1-2 minutes to remove excess of both stains. Blot out the ethanol. - Do not leave the section for too long in ethanol as it may remove too much of the stain. The section should appear translucent to blue. If it appears red, then the excess stain has not been effectively removed (this happens when the sections are too thick).
12. Gently transfer the section to a microscopic slide and with filter paper blot out excess ethanol.
13. Immediately add 1-2 drops of mountant (glycerol: water, 1:1) and add a coverslip over the section. Water can be used for mounting.
14. Label the slide and examine under low power (4x objective) and higher power (10x objective) of your microscope.

**Study under the microscope:** Focus the slide under lower of microscope and then change to high power if needed