Mounting procedure stained by Toluidine Blue and Safranin

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Microtoming

After the samples are embedded in paraffin, it is ready for microtome dissection. Stick the sample in a wooden block which has been soaked in paraffin before hand. For general dissection of plant tissue, 8-12.5μm thick is typical, but varies on different materials. Cut the ribbon in desired length and place on a warm water bath (55°C), this could be a Petri dish or a designed instrument. Use microscope slide to pull out the ribbon and place on the middle of the slide. Dry the slides on a warm plate (55°C) overnight.

Staining (followed Johansen, 1940)

To remove paraffin, follow the steps and transfer to next step every **5-10 mins**.

xylene
xylene + ethanol (1:1)
95% EtOH
70% EtOH
35% EtOH
dH_2O
dH_2O
Tol-Blue in dH_2O – or stain in other dye (**Safranin***)
dH_2O
dH_2O
35% EtOH
70% EtOH
95% EtOH
xylene + ethanol (1:1)
xylene

Take out the slide and add a drop of Permont, put on the cover glass and put a "weight" on the slide. The slide should be ready overnight.

***Safranin solution** (for 200ml)

dissolve 2g **Safranin O** in 100ml **methyl cellosolve** (=ethylene glycol)
When solution complete, add 50ml **95%EtOH** and 50ml **dH_2O**
Then add 2g **NaOAc** and 4ml **formalin**

Things I use in Mike's lab:

Microscope slide – Fisherbrand superfrost®/Plus, precleaned (Cat.#12-550-15)
Cover glass – Corning, No. 1, 22mm sq., FISHERfinest™ Premium cover glass
Microtome – Leica RM2065; and Leica disposable microtome blades (Model818)

Weight – West Coast premium magnum shot No. 9, West Coast Shot Inc., filled in a small glass vial (Vial, S/T, Type I glass, FISHER Cat.#03-338-25B, $105.3/144vial), but I think the cheaper one will work too (FISHER Cat.#03-377A $12.22/100vial)
Toluidine Blue O – Fisher (Cat.#BP107-10)
Safranin O – Sigma (No. S 8884)
Methyl cellosolve (=ethylene glycol=monoethyl ether) – Fisher (Cat.#E182-500)

Reference:
Johansen, D. A. 1940. Plant microtechnique. McGraw-Hill Book Company. New York.