

MOUNTING PROCEDURE STAINED BY PAS (PERIODIC ACID/SCHIFF'S) REACTION

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☉ **Microtoming**

(See Tol-blue mounting/staining)

☉ **Staining** (follows O'Brien & McCully, 1981 and Ruzin, 1999)

Once the paraffin-embedded sample is adherent to the slide, follow the steps and transfer to next step every **5-10 mins**, unless when specified.

xylene

xylene + ethanol (1:1)

95% EtOH

70% EtOH

35% EtOH

dH₂O

dH₂O

DNPH* in 15% acetic acid, **30min**

dH₂O

dH₂O (dry the slide if the sample falls off)

1% **periodic acid**, **10min**

dH₂O

Schiff's reagent[‡], **30min** (Reduce if too purple)

[0.5% **Sodium metabisulfite** in 1% HCl] 3 times, 2min each

dH₂O

dH₂O

Tol-Blue (you can omit or use other counterstain)

dH₂O

dH₂O

35% EtOH

70% EtOH

95% EtOH

xylene + 100%EtOH (1:1)[§]

xylene

xylene

A **Fast Green** counterstain can be used after 95%EtOH:
+**Fast Green** soln[†] for 15sec (up to 30sec if not green)
+100%EtOH, 2min
+100%EtOH, 2min
xylene + 100%EtOH (1:1)[§]
xylene
xylene

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

***DNPH** (2,4-dinitrophenyl-hydrazine) in 15% acetic acid: Drop only a little powder into 15% acetic acid, shake for 1-2 hours, until it is saturated.

‡**Schiff's reagent:**

Basic fuchsin	0.5g
Sodium metabisulfite	0.5g
0.15N HCl	100ml

Stir until totally dissolved. The color should be light brown-orange, you can add activated carbon if you want to get rid of the color, then you have to filter out the carbon afterward. You can test the solution by adding a drop of Schiff's reagent and a drop of formaldehyde, you should get a bright purple color.

†**Fast Green solution** (sensu Ruzin, 1999)

Methyl cellosolve	1vol
100% EtOH	1vol
Clove oil	1vol

- A **clearing solution** can be used for Fast Green, which is composed by 2vol of methyl salicylate (or clove oil) + 1vol 100%EtOH + 1vol xylene.

§ Alternatively, you can use xylene plus 2-3 drops of 100%EtOH, or a mixture of methyl salicylate and xylene (1:1) to remove the last trace of water.

☺ **Things I use in Mike's lab:**

Basic fuchsin

Clove oil

DNPH - Sigma (D-2630, 100g)

Fast Green -

Methyl cellosolve (=ethylene glycol=monomethyl ether). Fisher (Cat.# E182-500)

Periodic acid - Fisher (BP581-25)

Permunt - Fisher (Cat.# SP-15-100)

Sodium metabisulfite ($\text{Na}_2\text{S}_2\text{O}_5$)

Toluidine-blue O

Weight (for glass slide) – 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)

☺ **Reference:**

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

O'Brien, T. P. and McCully, M. E. 1981. The study of plant structure principles and selected methods. Termarcarphi Pty Ltd. Wantirna Victoria, Australia.

Ruzin, S. E. 1999. Plant microtechnique and microscopy. Oxford University Press. New York.