Feulgen staining, whole mount (Barrell 2005, modified):
- Povilus, Friedman Lab, 2014

- Dissect material and fix as appropriate. Place samples in 2 mL microcentrifuge tubes. For all steps, use ~1.5 mL solution or more. When samples are on the shaker table, periodically inspect to make sure that tissue isn't sticking to the tops/sides of tubes and not getting washed properly. Do not let samples dry out in-between steps. Incubation times (especially HCl) may require adjustments. Wash step number and times can be varied – times noted are minimum suggested times.

*note: if you are having a a problem with tissue sticking to pipette tips as you change solutions, use syringes with metal needles

- ☐ Start with samples in 70% Ethanol

- ☐ Wash in 50% Ethanol, on shaker table ~20 min at room temp
- ☐ Wash in 30% Ethanol, on shaker table ~20 min at room temp
- ☐ Wash in 10% Ethanol, on shaker table ~20 min at room temp

- ☐ Wash in water, on shaker table ~20 min at room temp

- ☐ 1 M HCl for 2-3 hours (change solution every hour if possible), on shaker table
- ☐ Wash in water, on shaker table 10 min at room temp
- ☐ Schiff's reagent, overnight (16 hours) at 4 C (can also try ~4 hours at RT)
- ☐ Wash in water, on shaker table 10 (or more) min at room temp
- ☐ Wash in water, on shaker table 10 (or more) min at room temp

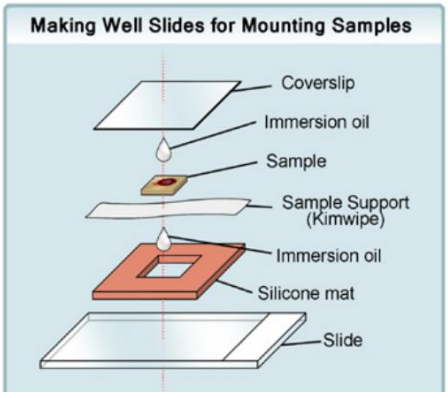
--- Dehydration/Infiltration:
- ☐ 10% Ethanol, 20 min @ RT on shaker table
- ☐ 30% Ethanol, 20 min @ RT on shaker table
- ☐ 50% Ethanol, 20 min @ RT on shaker table
- ☐ 70% Ethanol, 20 min @ RT on shaker table (can hold overnight)
- ☐ 85% Ethanol, 20 min @ RT on shaker table
- ☐ 95% Ethanol, 20 min @ RT on shaker table
- ☐ 100% Ethanol, 20 min @ RT on shaker table
- ☐ 100% Ethanol, 20 min @ RT on shaker table

<u>OPTION A (large/difficult samples):</u>
- ☐ 1:1 100% ethanol to JB4 Monomer A (with catalyst), ~30 min @ RT
- ☐ JB4 Monomer A (with catalyst). Date _____
- ☐ JB4 Monomer A (with catalyst). Date _____
- ☐ JB4 Monomer A (with catalyst). Date _____

Let sit in Monomer A for 1-2+ weeks, change Monomer A solution at least twice.

<u>OPTION B (easy samples):</u>
- ☐ 1:1 Immersol 518f (zeiss):100% EtOH, 20 min @ RT on shaker table
- ☐ 100% Immersol 518f (zeiss), 10 min @ RT on shaker table

Carefully remove samples (transfer pipette or very fine forceps) and mount on slides (may have to glue coverslip pieces to form a supported sample well, or use silicone mats) in immersion oil. *Tip: to help keep tissue from rolling around on the slide during observation, spread a little clear nailpolish on slide and let dry partially before adding tissue, or use a small strip of kimwipe as a hammock. Add coverslip (can seal with clear nailpolish).



Barrell, P and Grossniklaus, U. (2005) Confocal microscopy of whole ovules for analysis of reproductive development: the *elongate1* mutant affects meiosis II. The Plant Journal: 43, 309-320