Alexander staining

Note: The staining solution contains toxic chemicals. Handle it in the fume hood.
Reference: *Alexander MP (1969): DIFFERENTIAL STAINING OF ABORTED AND NONABORTED POLLEN. Stain Technology 44:117-122.*

A. Preparation of the Alexander staining solution

Add to a light protected bottle:
10 ml 96% ethanol
10 mg Malachite green (1 ml of 1% solution in 96% ethanol)
50 ml distilled water
25 ml glycerol
5 gm phenol
5 gm chloral hydrate;
50 mg acid fuchsin (5 ml of 1% solution in water)
5 mg Orange G (0.5 ml of 1% solution in water)

Glacial Acetic acid to the final concentration of 4%

Keep in dark

B. Sample preparation

1. under a stereomicroscope dissect flower that is about to open (opened flowers are too old) and place it into a drop (20 µl) of Alexander staining solution on a microscopic slide. Note, do not use flowers from very young or very old inflorescences as those may have some problems with fertility.

2. Cut the flower in the bottom 1/3 with scalpel and remove all tissues except for anthers using fine forceps. If the pollen sac already opened use younger flower. Anthers from several flowers can be put on a single slide.

3. Cover with a coverslip and add more staining solution if needed. Seal with the rubber cement (Fixogum) to prevent evaporation.

4. Let stain for several hours (7-8 h). Incubation at 50°C for several hours makes the staining faster. Note: too much staining solution or too long incubation may result in higher background signal that makes evaluation of staining more difficult.

5. Inspect under the stereomicroscope. Red or pink color indicates viable pollen while the green one non-viable.