Histochemical analyses of pollen grains and pollen tubes (Nishikawa et al 2005, BMC Plant Biol 5: 22)

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auramine O staining of pollen exine

1. Prepare a staining chamber on a slide glass using vacuum stop cock grease as shown in Fig 1.
2. Place 3 to 4 stamens in the center of the chamber and cover them with a piece of cover slip.

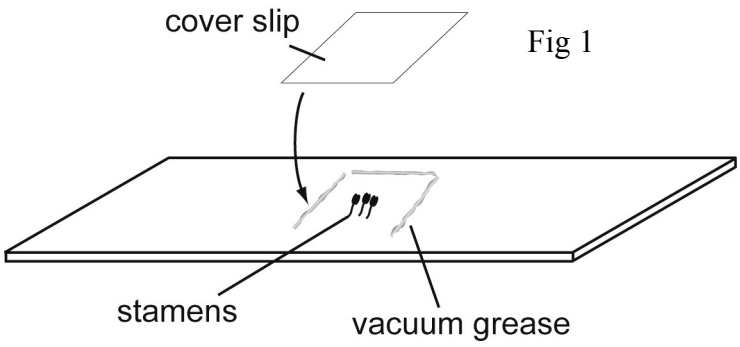

Fig 1

3. Add staining solution (0.01-0.1% auramine O in 50 mM Tris-Cl, pH 7.5) from the open side of the chamber and tap the cover slip with forceps to disperse pollen grains.
4. View under a confocal microscope (FITC or GFP setting).

Histochemical staining of pollen tube wall

Pollen tube growth

Pollen growth media

final conc.	stock	100 ml
18% sucrose		18 g
0.01% boric acid	4%	0.25 ml
1 mM $CaCl_2$	1 M	0.1 ml
1 mM $Ca(NO_3)_2$	0.1 M	1 ml
1 mM $MgSO_4$	0.5 M	0.2 ml
10 mM Hepes-KOH, pH 7.0	1 M	1 ml
0.5% agar		0.5 g

Heat to dissolve agar and pour into plastic Petri dishes (Falcon 3001, 4-5 ml/plate).

1. Dust pollen grains and incubate at 23°C for 6h to o/n.

2. Add pollen growth media (without agar) to germinated pollen tubes. Transfer the pollen tubes to polylysine-coated slides by touching the surface of media with the coated slides.
3. Stain pollen tubes with staining solution and view under a microscope.

Staining

Callose

Staining solution: 0.05% aniline blue in 50 mM K-phosphae, pH 7.5

Stain 5-10 min at RT and view under a fluorescent microscope with the DAPI filter set.

Cellulose

Staining solution: 0.01% calcofluor white (fluorescent brightener 28, Sigma) in 0.1 M K_2HPO_4

Stain 30-60 min at RT and view under a fluorescent microscope with the DAPI filter set.

Pectin

Staining solution: 1% alcian blue 8GX (Fluca) in 3% acetic acid

Stain ~60 min at RT and view under a bright field microscope.