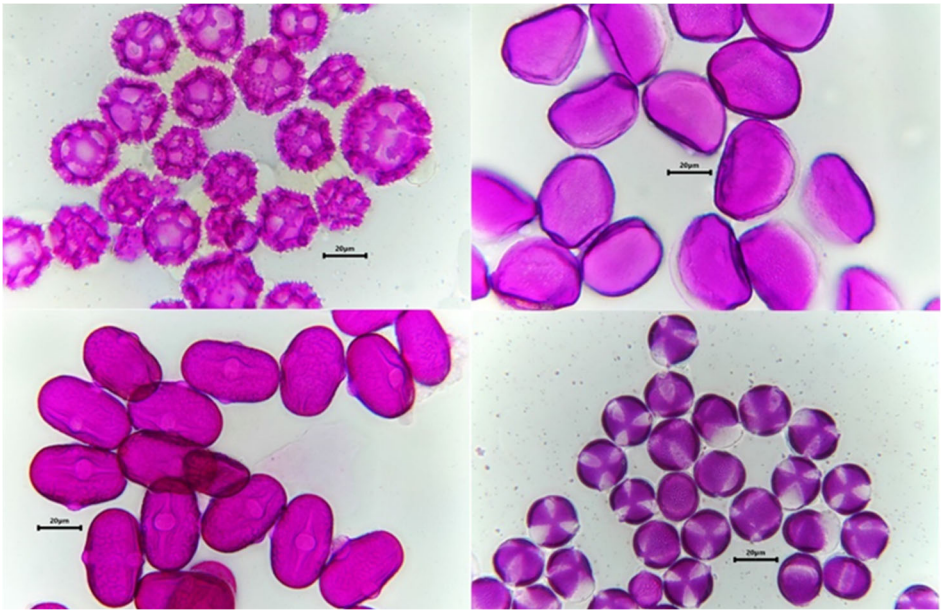
MY POLLEN PREPARATION MATERIALS AND METHODS
by Chris Thomas



17. Four different pollen types isolated from individual pollen pellets from a commercial superfood preparation. Top left: Dandelion. Top right: Bluebell. Bottom left: a legume (bean related) type. Bottom right: oilseed rape. Scale bar is 20 µm. Image stacks, 40x objective. Glycerine Jelly.

I have made several hundred pollen preparations and slides over the past year using a variety of sources, from pollen pellets to airborne samples. Here, I'd like to share what worked for me and the equipment I found useful.

Three important facts about pollen:

1. Most pollen is remarkably robust. It can withstand repeated hydration dehydration, and rehydration, and will survive in the environment for thousands of years under the right conditions, making it a useful tool for archaeologists.
2. Most pollen grains sediment in water or alcohol to form a nice pellet within an hour in a 30ml tube, or 20 mins in a 1.5 centrifuge tube.
3. Pollen grains can be easily stained and mounted for microscopy.

Armed with these facts, pollen preparations are one of the most accessible subjects for slide making.

MATERIALS

These are the materials I use; equivalents can be substituted:

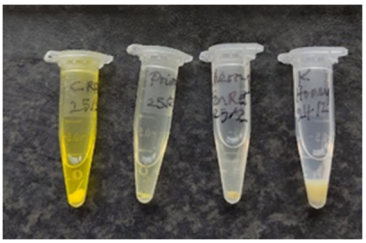
- Clean microscope slides and cover slips (I use 22 mm x 22 mm)
- Glycerol.
- Tap water for rinsing.
- Deionised water for suspending samples. (As sold for cars).
- Isopropanol or alcohol.
- Red glycerine jelly for pollen – from Brunel Microscopes or recipe below.
- OPTIONAL Basic Fuchsin stain (Brunel Microscopes).
- OPTIONAL Canada Balsam mountant.
- OPTIONAL LOCA or Vida Rosa UV resin setting gels and long UV lamps.
- Cheap nail varnish.
- Vinegar & tissues for cleaning slides.
- Washing up liquid.
- 30 cm x 30 cm square piece of netting for airborne pollen.
- Jam jar – for large sample volumes.
- 30ml specimen tubes for intermediate volumes, available in batches of 50 from Amazon.



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18. 30 ml specimen tubes with most supernatant removed – pollen still in process of sedimentation – see clearing of solution above sediment.

- 1.5 ml centrifuge or specimen tubes for small volumes, available in several hundred on Amazon.



19. 1.5 ml microcentrifuge tubes with pollen pellets.

- 3 ml and/or 1 ml plastic pipettes – available in batches of 50 from Amazon.



20. 3 ml and 1 ml plastic pipettes.

- DESIREABLE: warm plate to dry and mount pollen slides.
- OPTIONAL microcentrifuge – to pellet 1 ml pollen preps quickly at 7000 rm, 2680 x g, rather than by sedimentation.



21. 8 x 1.5 ml sample tube micro-centrifuge Camlab D1008.

METHODS COMMON TO SEVERAL PROCEDURES

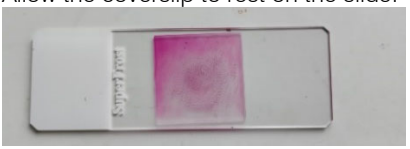
Pollen Sedimentation in Water or alcohol (NOT diluted honey).

- LARGE VOLUME (100 ml – 300 ml).
  - Place pollen suspension in jam jar overnight to settle.
  - Siphon or pipette off most of liquid without disturbing the sediment.
  - Resuspend sediment in remaining liquid and transfer to medium volume 30ml containers.
- MEDIUM VOLUME (10 ml- 30 ml)
  - Place pollen suspension in 30 ml tube(s). Leave 1 h to settle.
  - Pipette off most of supernatant, leaving about 1 ml above pellet.
  - Resuspend pellet in supernatant and transfer to 1.5 ml tubes.
- SMALL VOLUME (1.5 – 6 ml).
  - Distribute pollen suspension in one or more 1.5 ml tubes.
  - Allow pollen to settle for 20 minutes. Or spin balanced tubes for 30 seconds in microcentrifuge.
  - Remove most of supernatant.
  - Resuspend pollen pellet;
    - EITHER in 1 ml alcohol where cleaning indicated, followed by re-sedimentation for 20 minutes.
    - OR in deionised water allowing:
      - 1 ml for a substantial pellet (3-5 mm high in 1.5 ml tube).
      - 0.2 ml for a barely visible pellet.
      - Your best guess for pollen amounts in between.
    - OR dilute aqueous Basic Fuchsin stain – see later.
- ESTIMATING SEDIMENTATION TIMES
  Allow 1 minute for every 1mm of liquid height for aqueous pollen suspensions. Sedimentation in alcohol is faster.

STANDARD: Mounting pollen in red glycerine jelly pollen mountant

- Apply 100 μl (0.1 ml) of final pollen suspension to centre of slide and spread to still fit under your coverslip.
- Allow to dry, either at room temperature or on a warm plate (hand hot).
- Melt your red glycerine jelly pollen mountant hand hot. Warm slide.
- Add a small drop of the glycerine jelly to the centre of your pollen preparation with a thin glass rod pipette.
- 
  22. Pollen slide with re glycerine jelly drop.

- Place the edge of your coverslip on the slide to one side of the drop and gently lower till it contacts the jelly. Doing this carefully reduces the likelihood of bubbles being trapped.
- 
  23. Gently lowering the coverslip onto the glycerine jelly on the pollen slide.

- Allow the coverslip to rest on the slide.
- 
  24. Pollen slide with glycerine jelly and coverslip in place.

- If you have too little glycerine jelly to fully fill under the coverslip, add a drop at the edge of the slip and allow it to seep in.
- Leave the slide on a warm plate for 15 minutes.

- If it seems that the red stain is disappearing in the area of the pollen spread, this is the stain being extracted and binding to the pollen.
- Allow the slide to cool for gel to set.
- To remove excess jelly outside of the coverslip, place the slide under cold running water and gently brush, with an old toothbrush, along the edge to remove the excess. Then dry the slide.

- **OPTIONAL IF PROCESSING MANY SAMPLES SIMULTANEOUSLY: Instead of 0.1 ml drops, use much smaller drops and apply 5 different samples to a slide – 3 in a top row and 2 in a row below to aid orientation and identification of samples later. You will need longer cover slips; 22 mm x 40 mm coverslips work well.**

- **To make your mount permanent, seal along the edges of the coverslip with nail varnish, covering the coverslip with a mm or two of the varnish, I use clear varnish as it is less obtrusive.**

PROS: Glycerine jelly is the standard method that produces hydrated, expanded, stained pollen grains for identification.
CONS: prone to including bubbles. Gentle handling minimises this. Slides will dry out if not properly sealed.

ALTERNATIVE: Prestaining pollen

- Make a pale pink Basic Fuchsin*** solution in deionised water.



25. Dilute aqueous Basic Fuchsin stain

- Resuspend your pollen pellet from any one of the methods in the dilute stain.
- Take a small drop and check stained pollen grains visible under microscope. If
- NO allow pollen to settle. Take off supernatant resuspend in stain. Repeat until sufficient staining occurs.
- YES: spread 100 μl of the suspension on a microscope slide and allow to dry.
- Mount in undyed glycerine jelly for hydrated pollen, or a mountant for dehydrated pollen.

Making your own Glycerine Jelly

The original 1880 recipe by GJ. Kaiser is taken from Walter Dioni's article Part 4 of Mounting Microscopic Subjects, online at Micscape: https://bit.ly/glycerinejelly

- Water - 21g
- Glycerine - 12g
- Gelatine powder- 7g
- Listerine (antiseptic solution) - 2g
- Sprinkle the gelatine over the water-glycerine mix and leave it to soak for at least five minutes.
- Melt it over a low heat,
- Take off heat and add the antiseptic. stir very slowly to avoid air bubbles.
- Leave the flask in warm water for a while for the bubbles to disappear.
- Pour the medium into a wide mouthed bottle."
- If desired, you can add Basic Fuchsin*** stain – see below.

Basic Fuchsin*** Stain stock

- Dissolve 50 mg of dry Basic Fuchsin crystals in 5 ml isopropanol.
- Add drops of dye to 10 ml of deionised water, or to glycerine jelly, until you achieve a pale pink colour. Try it out first. You can always add more dye later.

***BASIC FUCHSIN is regarded as a possible carcinogen by some authorities. See handling and safety notes on page 5.

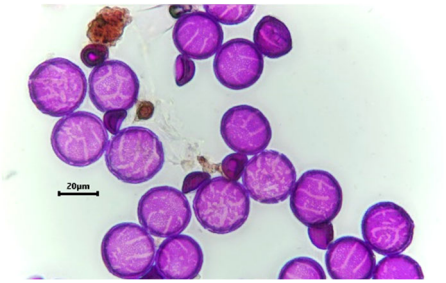
SPECIFIC POLLEN COLLECTION METHODS

Pollen directly from single flower

- Take a very small fragment of pollen glycerine jelly and rub into a smear on a microscope slide.
- Expose the ripe anthers of a flower and tap onto the smear on the microscope slide.
- Mount sample: add a drop of red glycerine jelly for pollen and cover slip as in standard method above.

PROS: Quick and simple – good for sampling in the field.
CONS: May get obscuring clumps of pollen with fewer individual grains. Pollen oils may obscure surface structure.

Pollen from rinsed flowers

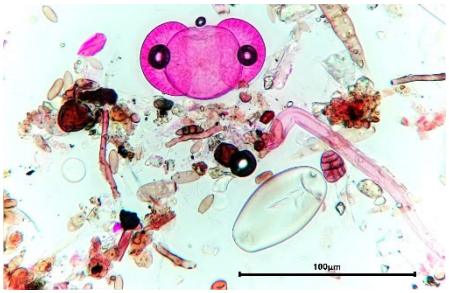


26. Stack of primula pollen grains. 40x objective. Glycerine Jelly.

- Use flowers trimmed down to ripe anthers or use isolated anthers.
- Dip and shake into alcohol solution in:
  - 1.5 ml tubes for pollen rich anthers.
  - 30 ml tubes for anthers with little pollen. Dip several flowers from one species into the same container.
- Remove any visible plant debris or broken off anthers in the liquid with a toothpick or forceps.
- Allow pollen to sediment, with a final pellet in 1.5 ml tube.
- Add appropriate amount of deionised water.
- Mix to suspend pellet evenly.
- Apply, dry and mount sample in glycerine jelly for pollen. – see Mounting methods.

PROS: Gives good clean pollen spreads. To adjust amount loaded, you can dilute concentrated more or allow dilute samples to pellet to resuspend in smaller volume.
CONS: Dealing with liquids and waiting times for pollen to settle.

Airborne pollen and fungal spores



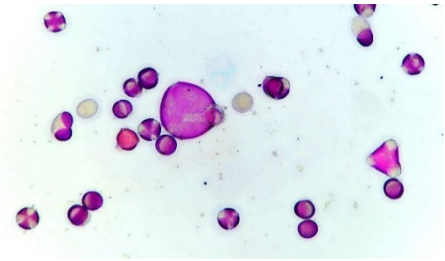
27. Airborne pollen, spores, starch grains and debris. 10x objective. Note bubbles, AKA focussing aids! Glycerine Jelly.

- Slightly moisten a 30 cm x 30 cm piece of netting with glycerol.
- Hang up on washing line with pegs and leave for 4 hours to overnight.
- Place netting in a standard jam jar and add water plus a few drops of washing up liquid to almost fill jar.
- Screw on lid to jar and shake vigorously.
- Carefully draw out netting, whilst squeezing out liquid into jar.
- To remove foam, dribble a few ml of alcohol down the inside of the jar until the foam completely disappears.
- Replace the lid on the jar and allow to settle overnight.
- Follow Pollen sedimentation above, through to pellet in 1.5 ml tube.

- Mount sample in pollen glycerine jelly mount – see Mounting methods.
- OPTIONAL To remove oils, remove supernatant and resuspend pellet in 1 ml alcohol, then let settle again for 1 h, or spin for 30 seconds, before resuspending in water and going through mounting procedure.

PROS: Gives good spreads with amazing amounts of material in spring and summer.
CONS: Dealing with liquids and waiting times for pollen to settle.

Pollen from Honey – (needs longer initial sedimentation time)



28. Mix of pollen grains from a predominantly oilseed rape, smooth flow honey. 10x objective. Glycerine jelly.

Method adapted from Melissopalynology: Pollen Analysis of Honey, by John Rhodes.

- Stir your honey to resuspend any pollen. If crystalline solid, warm to about 37°C until honey liquifies.
- Mix 10 g to 50 g of honey with 5 volumes (50 ml to 250 ml) warm water in a suitable jam jar, ensuring no sugar crystals remain.
- LEAVE OVERNIGHT .
- Transfer to 30 ml tube LEAVE OVERNIGHT.
- Resuspend sediment in 20 – 30 ml deionised water in the 30 ml tube (we want to dilute out any remaining sugar) and allow to sediment at least 1 hour.
- resuspend pellet in 1 ml deionised water. Transfer to 1.5 ml tube.
- Mount pollen – see methods.
- If sample too dense, dilute in more deionised water. If too dilute, allow to settle and resuspend pellet in smaller volume of deionised wate and try again.

PROS: Works well with most honeys.
CONS: Liquid handling and two overnight precipitation times.

Pollen from Honeybee pollen pellets

- Place a pollen pellet in a 1.5 ml tube.
- Add 0.4 ml deionised water and leave to soften for 5 to 15 minutes.
- Resuspend pellet in liquid by shaking closed tube or gentle pipetting.
- Add 0.4 ml alcohol, mix, and allow to settle as for Small Volume sedimentation above.
- Resuspend pellet in 1.5 ml tube in 1 ml deionised water.
- Mount pollen – see methods.

PROS: This is a fast and simple method that gives good clean pollen spreads with most pollen pellets. I've processed 40 to 60 individual pellets in an afternoon. See first figure of article.
CONS: None really.

Pollen microscopy

- Scan your slides with a 10x objective to get a feel for the distribution, the variety or uniformity of different pollen grains, and also the variations in stain.
- Once you have an overview, centre a promising view of pollen grains that are not over or under stained.
- Switch to a 40x objective 0.65 NA. This is generally adequate to see the pollen grain detail. Most grains are in the range of 20 to 50 μm diameter.

- Focus on the top surface to see if there is any surface structure.
- Then focus on the equator.
- Look at a range of pollen grains and orientations from the same type.
- For detailed structure, you will need to use an oil immersion objective. I prefer my 45x 0.95 NA oil immersion objective for most work.
- After oil immersion, clean slides with washing up liquid and COLD water.
- I photograph a focus stack and use the free Picolay for image stacking.

MOUNTANTS FOR DEHYDRATED POLLEN GRAINS.

PROS: Simple permanent mounts.
CONS: Dehydrated pollen grains may appear smaller with less visible surface detail than hydrated pollen at equivalent magnification- see figure below for comparison.

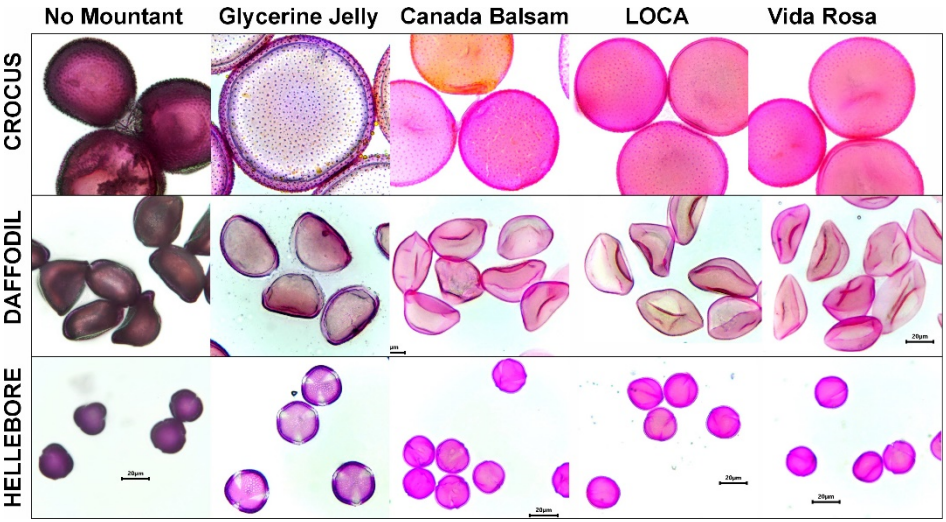
Mounting in Canada Balsam

- Add a drop of Canada Balsam to centre of dried, stained pollen on slide.
- Add a cover slip and leave on a warm plate till Canada Balsam fills coverslip.
- Slide can be viewed immediately but best left overnight on warm plate or longer for balsam to set when cooled.

Mounting in LOCA or Vida Rosa resin UV setting gels

- Add drop of LOCA or Vida Rosa on centre of dried stained pollen spread.
- Add a cover slip and allow to spread under slip.
- Set the gel for 60 seconds, using either a long wave UV torch (Pee Light) or a manicurists UV light for setting nails.

Chris Thomas
chris@miltoncontact.com



29. Comparison of pre-stained pollen preparations from Crocus, Daffodil and Hellebore in different mountants at identical scale. Glycerine jelly gives hydrated expanded pollen grains. All other methods show dehydrated unexpanded pollen. 40x objective.

FROM THE WEBMASTER– Peter Wyn Jones

March 2024 Website: https://www.quekett.org

The website continues to attract 100-200 hits per day. Although you might look at the Programme page for details of upcoming events, have you ever surfed through the other pages to see what they have to offer?

The Resources page (https://www.quekett.org/resources) has a wealth of information on historical and technical aspects of microscopy ranging from production records of some of the principal manufacturers in the Little Imp Archival Series to how to set up Kohler illumination, and how to make Rheinberg discs. There are also details of suppliers of used microscopes and firms who offer microscope servicing.

The Microscopy for Beekeepers page (https://www.quekett.org/resources/beekeeping) is a useful place to start for those who may be considering studying for the certificates associated with this increasingly popular activity.

The technical pages have been put together by members of the Club and so reflect the enthusiastic amateur approach of our society. There is always something new to learn and we are keen to add to the fund if information on the resources page, so welcome any contributions you may have. Take a look at the page to see what you can do.

The pages linked to Club membership (Joining the Quekett and Manage My Membership, https://www.quekett.org/about/manage-membership) have now bedded in well and have been welcomed by both the membership and the committee members involved. The SheepCRM software which supports the process seems to run smoothly and so the change from the old system appears to have been a sound investment. You can check your current details in SheepCRM through the link on the above page.

Personal microscopy has focussed on slimming down the collection of incidental bits and pieces which 'may come in useful'. Among the larger items I have is a Leitz Orthoplan which belonged to Klaus Kemp which I will have to move on as it is too big. I suspect moves like this are not uncommon as space for most of us is always at a premium. Although we do not have a section on the website for members to sell their offerings but the two Quekett sales events, in March (Reading) and August (Letchworth) are always well attended, and the Members' Auction (on-line) offers another route.

BASIC FUCHSIN SAFETY

Like any stain, Basic Fuchsin has to be handled with care and disposed of properly, especially in dry form, as it is regarded as a potential carcinogen in humans, a skin and eye irritant. Wear personal protective equipment (e.g. apron, gloves, eye protection)

- Check the suppliers Material Safety Data Sheet (MSDS) and your local environmental requirements. Fortunately, we use stains in very small amounts.
- Dry powder – regard as hazardous waste. Use absolute minimum or avoid.
- 1% solution –regard as hazardous waste. Easier to handle and control. Small quantities (10 ml) can be inactivated with concentrated bleach.
- Dilute stain solutions – can be inactivated with concentrated bleach.
- Sealed red pollen jelly slides – store safely. Label any solid waste container.