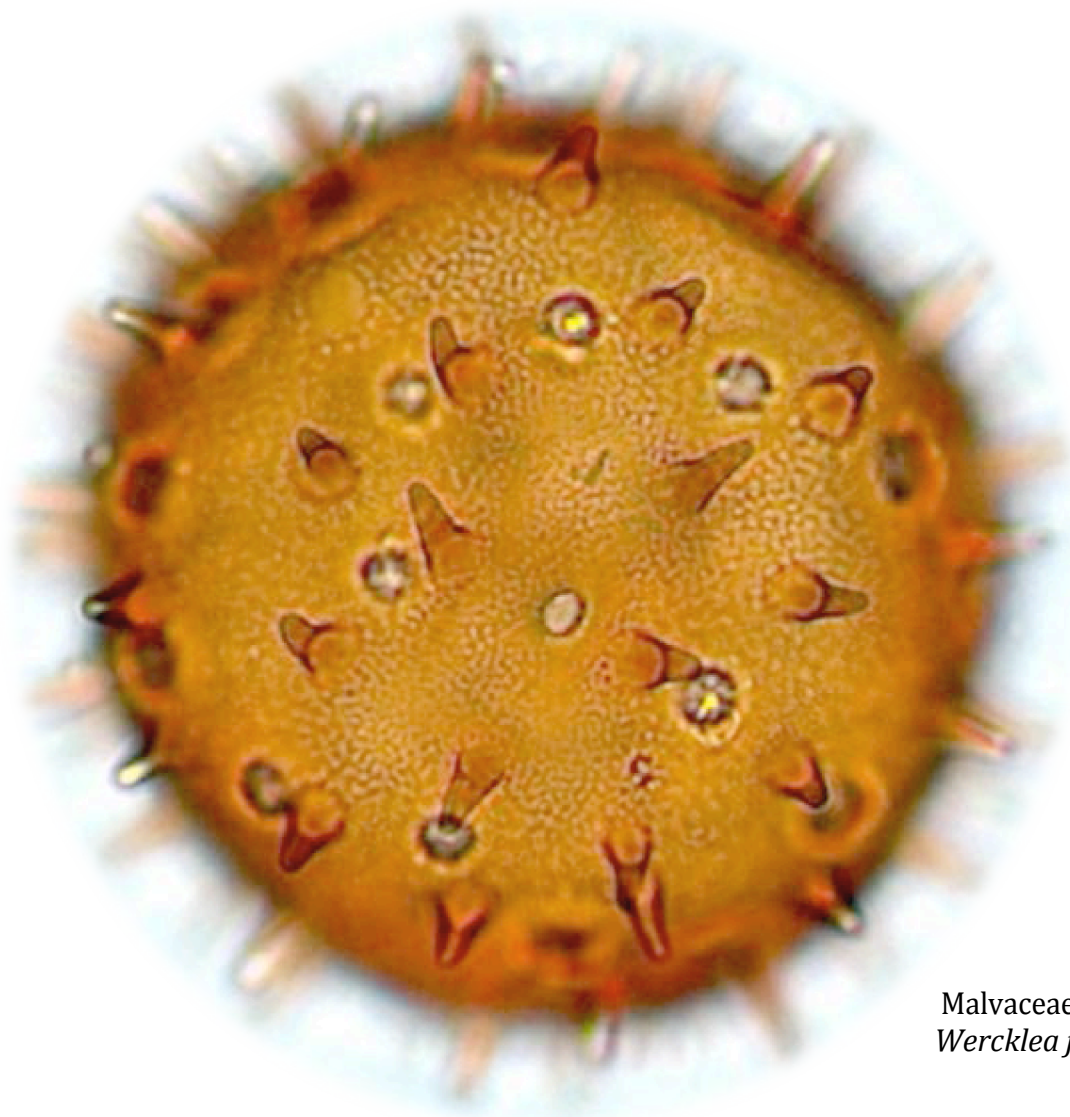


# Pollen Processing Reference Manual

Revised October 2014



Malvaceae:  
*Wercklea ferox*

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# Herbarium Sample Collection Protocol

## Materials:

- List of desired taxa
- Shovel-end forceps
- Fine tip forceps
- Log sheet
- Herbarium specific rules
- Cleaning cloth
- Camera
- Large storage bag
- Collection envelopes or bags
- Labels with collector information
- Permanent marker
- Magnifying glass

**Note 1:** Each herbarium will have its own set of specific rules and guidelines. Be sure to request a copy of these instructions prior to collecting.

**Note 2:** It is good practice to do research about the herbarium prior to the scheduled visit. Be sure to know the names of any contacts at the herbarium before arriving.

**Note 3:** Practice good collecting etiquette for the duration of the visit. Use proper manners, dress, and collection protocol. It is also important to wear comfortable, supportive footwear.

**Note 4:** Out of respect for the herbarium and staff members, headphones should be worn at all times when listening to audio devices.

**Note 5:** Carefully handle all folders and sheets at the herbarium. This is vital. Be sure to maintain the correct order for folder and sheet stacking. Always keep the folders and sheets horizontal (with plant material facing up), even when searching through a specific family. This horizontal position must be maintained at all times, especially when transporting the material to and from the workspace.

**Note 6:** Never remove any plant material from a sheet with a single flower.

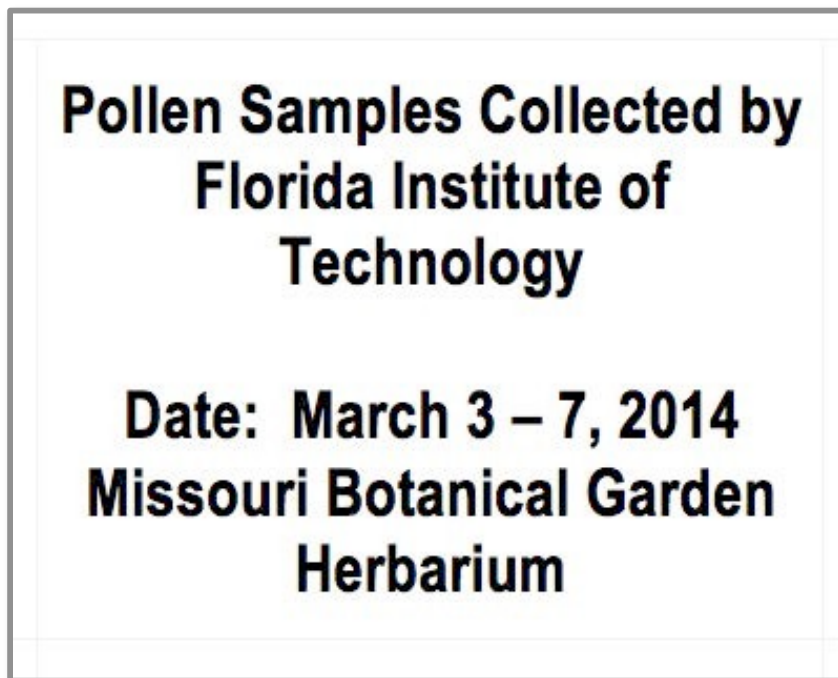
**Note 7:** As of 2014, small leaves, stems, bark, and other plant material should also be collected, in case of future phytolith processing.

**Note 8:** If any plant material comes loose from the herbarium sheets during collection, it should be placed in the fragment packet on that specific sheet. If there is no fragment packet on the herbarium sheet, request one from the herbarium staff.

**Note 9:** Only collect plant material from a single herbarium sheet. Examine several sheets within a folder before deciding which specimen is most likely to yield abundant pollen.

## **General Herbarium Rules:**

1. Restrict sampling to non-type specimens only. If you are unsure about the location or designation of the type specimens at a certain location, be sure to ask the herbarium director for further clarification regarding their type specimens.
2. Sample only from one or two species per taxon, unless species-specific collection is required.
3. Check the fragment packet for loose plant material, prior to collecting directly from the sheet.
4. Remove flowers only from herbarium sheets with ample flower material. As previously stated: Never remove the flower from a sheet with a single flower.
5. Place a label with the collection information on the herbarium sheet after removing the plant material. This label should include by whom the material was collected, purpose, date, & location (see Figure 1).



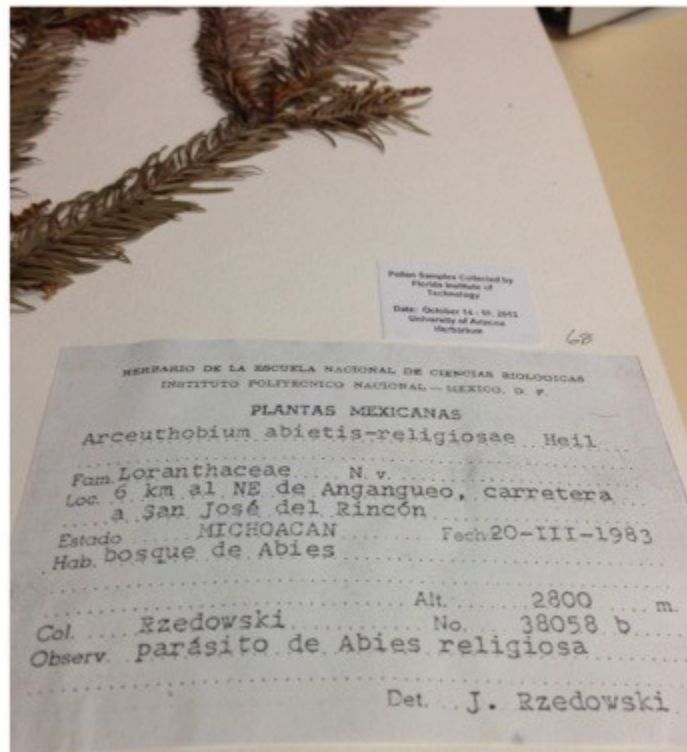
**Figure 1:** Herbarium sheet label

## **Collecting Samples:**

1. Reference the taxa list or priority list to determine which genera need to be collected.
2. Search the herbarium collection for the specific genus to be collected. The cabinets are typically categorized by family, then alphabetically by genus and species.
3. Remove the necessary folder(s) gently from the cabinet. Be sure to notate the exact location from which the file was removed, so it can be returned to the proper place after collection.
4. Remove the folder and carry it in a horizontal position to the workspace. Place the folder on a flat surface for collection. The folders and sheets should never be “flipped over” from their original orientation. They should be returned to the exact same stacking order after collection.



5. Search the sheets in the folders for one with sufficient flower material for collection, preferably from the fragment packet. After examining a sheet, place it off to the side of the folder. Maintain the proper stacking order so that the sheets can be returned to their folder in the appropriate order. Once again, do not flip over the sheets. **THIS IS VERY IMPORTANT.**
6. Check the fragment packet for material prior to sampling directly from any sheet. Try to find an ideal specimen with material in the packet. Only collect from the sheet directly if there is no material in the fragment packet on any of the sheets within that specific genus.
7. If there is material within the fragment packet, use the fine tip forceps to remove any whole flowers and place them into the collection envelope. If there is loose or broken flower material, use the shovel-end forceps to scoop the material and place into the envelope. If there is plenty of material available, multiple flowers can be collected. If there is no material in the fragment packets, use the fine tip forceps to remove one or two flowers from the sheet. **DO THIS VERY GENTLY.** Do not destroy or damage any of the other plant material on the sheet. If necessary, use the shovel-end forceps to gather any loose material. If the flowers are large, strictly anthers should be removed and placed in the collection envelope. Use the best judgment possible when determining whether or not the entire flower or just the anthers should be taken.
8. After collecting from an ideal specimen, label a collection envelope with the family, genus, and species.
9. Photograph the herbarium sheet description label for future reference (see Figure 2).



**Figure 2:** Herbarium sheet photograph for future reference

10. Seal the envelope.
11. Record the necessary information on the herbarium log sheet (see Figure 3).



# **Flower Preparation Protocol**

## **Materials:**

- Fine tip forceps
- Shovel-end forceps or Scoopula
- 1 mL Glass tubes
- Parafilm
- Gloves
- Water
- Permanent marker
- Flower material
- Micropipette (200-1000  $\mu$ l)
- Test tube racks
- 5 mL Travel tubes with modified lids (see Figure 1)
- Paper towels
- Protocol checklist
- Large magnifying glass with light
- Glacial acetic acid
- Glass micropipette tips

**Note 1:** There should be two sets of 24 tubes for each batch. Both sets will be prepared identically. After processing, one set will be used in mounting for light microscopy and the other set will be used for SEM photography. This will allow for ample pollen for each method.

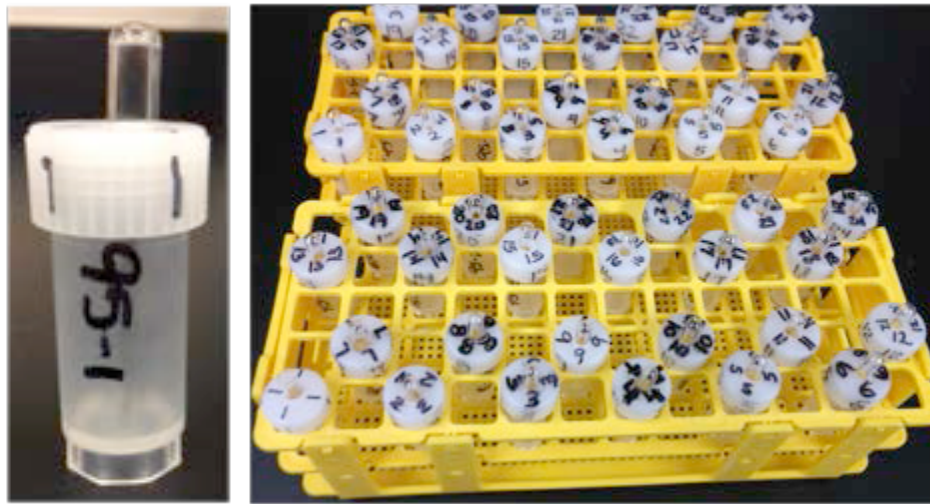
**Note 2:** The flower material may need to be broken up in order to fit ideally into the tube. If so, try to break the material as little as possible to minimize debris. Also, if it is necessary to break up the material, try to do so within the tube to capture any pollen that may come loose from the flowers.

**Note 3:** If possible, only use a portion of the flower material in the packet. Any remaining material can be used for second preparation, if necessary.

**Note 4:** Prior to the first preparation of the day, be sure to thoroughly wash all tools to ensure that they are clean before the preparation begins. If there is any doubt about the cleanliness of the tools or workstation, thoroughly re-clean everything.

## **Tube Preparation:**

1. Label the two sets of 24 plastic tubes with the processing code number with a permanent marker. Each tube should be labeled with the batch number and the tube number. For example Batch 75, Tube 3 would be labeled with code 75-3. This method should be used for labeling all of the tubes in the batch (see Figure 1).



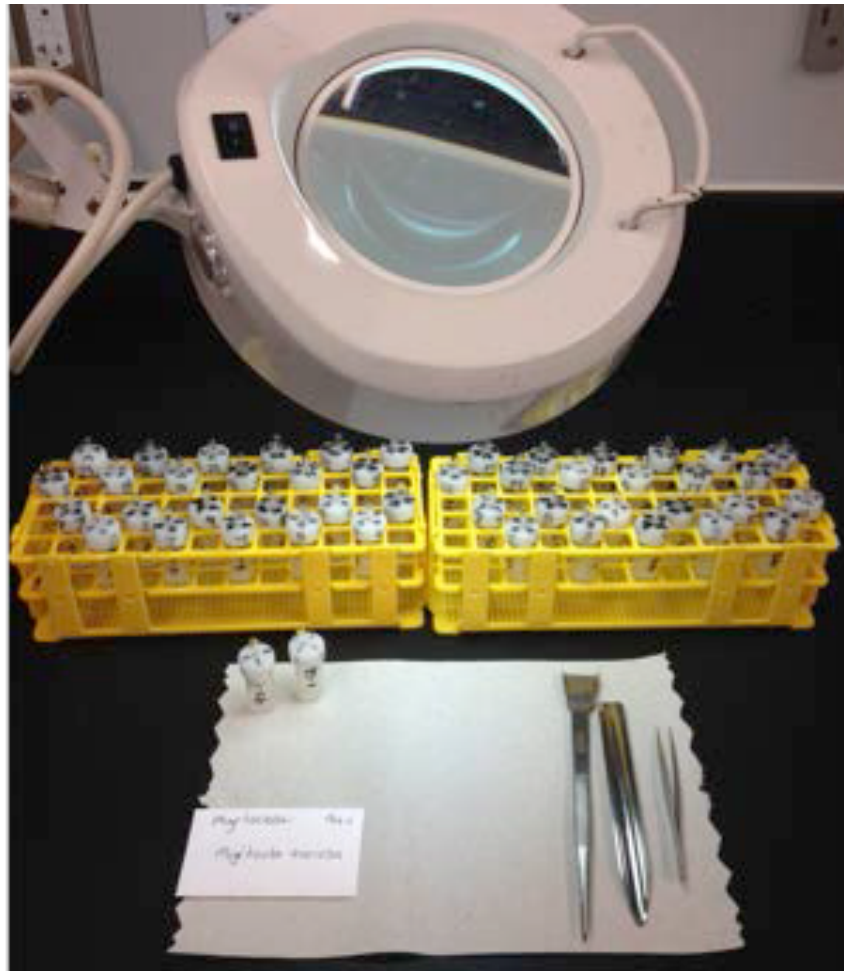
**Figure 1:** Left: Example of numbered tube, Right: Test tube rack set up

2. Place a lid with a corresponding number for that sample onto each tube. Lids are numbered in the general format of 1 through 24 to allow for reuse. There should be 24 samples per batch, prepared as two sets.
3. Place a clean 1 mL glass tube upside down into the numbered tube.
4. Put each set of tubes (numbered 1 through 24) into the test tube rack, leaving a vacant space in between each tube (see Figure 1).

### **Flower Preparation:**

1. Be sure the workstation is clean and free of open air flow.
2. Gather a group of 24 samples to process, typically categorized by herbarium, family and then genus. Try to use samples from the same family. Work through an entire family before starting the next. If there are not enough samples from a single family, use multiple families. Try to keep all of the samples from a single family together throughout processing.
3. Write the batch “prep” number, the family, as well as the genus and species for each sample on the ‘POLLEN PREPARATION PROTOCOL’ sheet.
4. Select the first sample and label the outside of the envelope with the appropriate code number. Write the genus and species of the sample onto the first available space on the protocol sheet. Wear gloves from this step forward.
5. Place a clean paper towel on the counter of the workstation. Adjust the magnifying glass to the appropriate level for viewing. Place both of the corresponding numbered tubes onto the paper towel for ease of access (see Figure 2).
6. Open the envelope containing the flower material and dump the contents onto the clean paper towel.





**Figure 2:** Workstation set up

7. Examine the contents. Search for anthers or flower material that is most likely to contain pollen. If the flowers are very small, the entire flower can be placed into the tube. If the flowers are large, place anthers and small portions of plant material into the tubes. If whole flowers or anthers are not present, use any plant material in the packet that may have pollen on it.
8. Gather the desired material with the metal tools and place into the glass tube. Equal amounts should be placed into each of the two tubes. Each tube should be filled no more than 1/2 full. An ideal tube is approximately 1/3 full.
9. Place the tube with plant material back into the test tube rack.
10. Cover the tube with small piece of parafilm or a clean paper towel to prevent contamination.
11. Place the remaining flower material back into the envelope and reseal it with the use of labels or tape. Set the envelope aside to store with the samples that have been processed.

12. Throw away the contaminated paper towel.
13. Wash all tools and gloves. Dry thoroughly.
14. Repeat this processing for the remaining samples in the batch.
15. Place the stack of plant material envelopes into the proper storage container for future use.
16. Wipe down the entire workstation with damp paper towels to remove any plant material that may have contaminated the area.

### **Adding the Glacial Acetic Acid:**

1. Transport the prepared tubes to the fume hood, keeping the tubes covered to prevent contamination.
2. Uncover the tubes.
3. Using a micropipette, place 400  $\mu$ l of 100% glacial acetic acid into each tube. Be careful not to let the glass tip of the micropipette touch the inside or outside of the glass tube. If the tip touches the tube, replace it with a clean tip to prevent contamination.
4. Thoroughly wrap each tube with parafilm.
5. Allow the samples to sit overnight before processing.

# Acetolysis Protocol

## Materials:

- Herbarium samples
- Permanent marker
- 1.7 mL Microcentrifuge tubes
- Test tube racks
- Glacial acetic acid
- Concentrated sulfuric acid
- Acetic anhydride
- 10 mL Graduated cylinder
- Heat block with aluminum element for 1.7 mL tubes
- 100 - 1000  $\mu$ l Pipette tips
- Centrifuge with inserts for 1.7 mL tubes
- Waste beaker
- 200  $\mu$ m Nylon mesh filters
- 200  $\mu$ m - 1000  $\mu$ m Micropipette
- Glycerol
- Safranin
- 10 mL Pipetter
- 10 mL Pipette
- Vortex
- DI Water in wash bottle
- 95% ethanol

## Filtering:

1. Place the filters inside microcentrifuge tubes, which then need to be placed into larger "buckets" (see Figure 1). Individual bucket assemblies are placed in the test tube rack next to each pollen sample, so each sample is paired with its own bucket assembly.
2. Remove the parafilm.
3. Vortex the glass tube until the solid and liquid contents are well agitated.
4. Pour the contents of the tube into the bucket assembly. Removal of all liquid may require "tapping" of the glass tube.
5. Add 300  $\mu$ l of glacial acetic acid (100%) to the glass tube.
6. Vortex the tube again and pour the liquid into the bucket assembly.
7. Fit the micropipette with the filter extension and main filter piece.
8. Draw the contents of the plastic tube up and down slowly several times to dislodge any remaining pollen from the filter. Drawing the liquid should be done slowly to avoid splashing liquid onto or into the end of the micropipette.
9. Remove the filter and place it in a waste beaker for washing.
10. Cap the microcentrifuge tube. A total volume of 700  $\mu$ l should be present in the plastic microcentrifuge tube.
11. Repeat this process until all samples have been filtered.
12. Close the lid on each of the samples.
13. Place clean plastic micropipette tips into each bucket (Figure 2).



**Figure 1:**  
Bucket assembly



**Figure 2:** Test tube rack setup with clean pipette tips

### **Preparing the Samples for Acetolysis:**

1. Centrifuge the samples at 2,000 rpm for 3 minutes to allow pollen to form a pellet at the bottom of the tube. If pollen sticks to the side of the tubes, rotate the tubes 180 degrees and re-centrifuge for 30 more seconds.
2. Fit the micropipette to the clean, plastic pipette tip that corresponds to the sample being drawn out.
3. Draw out the liquid from the tube and deposit it into a waste beaker. Draw out as much liquid as possible without disturbing the pellet. If the pollen is disturbed, return the liquid to the tube, re-centrifuge and draw out again.
4. Return the pipette tip to its bucket and attach a new tip.
5. Repeat this process until the liquid has been removed from all of the samples.

### **Preparing the Acetolysis Solution:**

1. Add the necessary amount of acetic anhydride to a 10 mL graduated cylinder using a quick release pipette pump fitted with a DRY 10 mL glass pipette tip. The sulfuric acid and acetic anhydride must be used in a 9 (acetic anhydride) to 1 (sulfuric acid) ratio. It should be calculated based on the number of samples being processed.
2. Carefully remove the tip and rinse with glacial acetic acid over the waste beaker.
3. Fit a micropipette with a DRY glass pipette tip to add sulfuric acid to the graduated cylinder containing acetic anhydride.

**Caution: Tips MUST be dry. Water reacts violently with the acids and may cause bodily harm.**

4. Add the necessary amount of sulfuric acid. Be sure to follow the 9:1 ratio mentioned above.
5. Gently release the pipette tip into the graduated cylinder for use in the next step.



## Acetolysis:

**Caution: The Acetolysis process must be performed in a water-free environment. Be sure to remove any water containers from the fume hood prior to starting this process.**

1. Uncap all samples being processed for a rapid addition of Acetolysis solution. The samples can be prepared 6 or 12 at a time depending on the user's preference.
2. Fit a micropipette to the glass tip from the graduated cylinder containing the Acetolysis solution. *Acetolysis is time sensitive. Reaction occurs immediately upon addition of the acids, so be prepared to work quickly.*
3. Add 300  $\mu\text{l}$  of Acetolysis solution to each tube being processed and immediately transfer to the heat block. As mentioned above, it is best to work in batches of 6 or 12 samples.
4. Return the tip to the graduated cylinder and loosely cover the graduated cylinder with a small piece of parafilm.
5. Place the tubes into the heater in the order by which the acid solution was added (see Figure 3). During this step, **leave the caps OPEN**. Add and remove samples in the same order that they were placed in the heat block. The first sample into the heater must be the first sample removed from the heater.
6. Keep the samples in the heating element for 3 minutes. The time may need to be adjusted, depending on the type of heater. Check the samples throughout this step by gently lifting them out of the heating element. Finished samples should appear light brown in color (see Figure 4).
7. After approximately 3 minutes, remove the samples from the heater and return to the test tube rack.
8. Using a clean glass pipette, immediately add 300  $\mu\text{l}$  of glacial acetic acid to all heated samples to stop the reaction.
9. Cap all finished samples and vortex thoroughly.
10. Repeat the Acetolysis procedure with the remaining samples.
11. Once all samples are completed, turn off the heater and put away acetic anhydride and sulfuric acid containers.
12. Rinse all tips and glassware with glacial acetic acid, and dispose of the waste in an approved and labeled waste disposal container.
13. Glassware can now be safely washed with soap and water.



**Figure 3:** Heating of samples during Acetolysis



**Figure 4:** Color change after 3 minutes

## **Water Wash:**

1. Centrifuge the samples for 2 to 4 minutes at 2,000 rpm.
2. Using the tips from each bucket, draw out the liquid from each tube and deposit into a waste beaker.
3. Rinse off the tips and accompanying buckets with water over the waste beaker. Return to the test tube rack.
4. Using a clean, glass pipette tip, add 500  $\mu$ l of water to each tube.
5. Cap the tubes, then vortex and centrifuge them.
6. Once again, wash all tips and buckets and return them to the test tube rack.
7. Using the rinsed plastic tips, add 500  $\mu$ l of water to the tubes. Plastic tips no longer need to be washed between each step.
8. Repeat this water wash process two more times with 500  $\mu$ l of water. A total of four complete washes are required. Washes can be recorded on the preparation protocol sheet.
9. After the final wash, vortex and centrifuge the samples.
10. Draw off the water down to the pollen pellet.
11. Properly dispose of the waste by placing it into an approved waste container.

## **Addition of Glycerol:**

1. Fill each sample to the 100  $\mu$ l (0.1 mL) line on the plastic tube with water.
2. Vortex the tubes, then uncap them.
3. Add one drop of glycerol with safranin and one drop of plain glycerol to each tube. Be careful not to drop the glycerol down the side of the tube. The viscosity may inhibit the stain from reaching the pollen at the bottom. If this occurs, add another drop of the required liquid into the water at the bottom of the tube.
4. Re-cap all tubes. Vortex for 10 seconds.
5. Place tubes back into the test tube racks and leave them to rest over night (see Figure 5).



**Figure 5:** Sample with glycerol and safranin

## **Ethanol Washes:**

1. After allowing the samples to rest overnight, add 500  $\mu$ l of ethanol to each sample tube.
2. Vortex each sample thoroughly.
3. Centrifuge for 2 to 4 minutes at 2,000 rpm.
4. Use plastic pipette tips to draw each sample down to the pellet.
5. Repeat this washing procedure twice more to ensure the removal of all glycerol.
6. Using a clean glass pipette, add ethanol to the samples to fill to the 100  $\mu$ l (0.1 mL) mark on the tube.
7. Re-cap the samples and place in the proper storage box for mounting and/or SEM photography.

# Pollen Slide Mounting Protocol

## Materials:

- Syn-Matrix mounting medium
- Microcentrifuge
- Microscope slides
- Slide coverslips (18mm x 18mm)
- Coverslip podium (see Figure 1)
- Capillary tubes
- Dissecting microscope
- Petri dish
- DI water bottle
- Kim wipes
- 250 mL waste beaker
- Permanent marker
- Light microscope
- Labels
- Trays for transportation
- Slide boxes
- Processed samples



**Figure 1:** Coverslip podium

**Note:** To make glass tools from capillary tubes, melt the tube into 1 of the 2 following formations:

**Round-tip tool:** This tool can be formed by placing the end of a capillary tube into an open flame. By gently turning the tool, a bulbous end will start to form (see Figure 2A). Ensure the glass closes completely before removing it from the flame. Allow the tool to cool completely before use.

**Needle-tip tool:** This tool can be formed by placing the middle of a capillary tube over an open flame and slowly pulling the two ends apart. The result will be a long, thin glass section that resembles a tip of a needle (see Figure 2B). Allow the tool to cool completely before use.



**Figure 2A:** Round-tip glass tool

**Figure 2B:** Needle-tip glass tool

## Pre-mount Preparation:

1. Retrieve the Syn-Matrix mounting medium from the refrigerator. Store the Syn-Matrix in the refrigerator when not in use to prevent the denaturing of the medium.
2. Place the first 6 samples in the microcentrifuge and ensure the samples are balanced within the device (see Figure 3). If they are not balanced, add ethanol to the samples until all of the samples contain the same amount. Also make sure all of the caps are placed into the machine in the same orientation.



**Figure 3:** Tubes in microcentrifuge

**Caution: Never run the centrifuge if it is unbalanced. It may cause damage to the machine or samples.**

3. Close the lid and allow the microcentrifuge to run at full speed for 10-20 seconds.
4. While the microcentrifuge is running, place a coverslip on an elevated podium (see Figure 4).
5. Place a petri dish upside down on the stage of the dissecting microscope to obtain a better viewing distance (see Figure 5).
6. Let the device stop completely before opening the lid. The pollen should be concentrated at the bottom of the tube. If it is not, the samples may be centrifuged again for a longer period of time.



**Figure 4:** Coverslip on podium



**Figure 5:** Petri dish on dissecting scope stage

## **Mounting Samples:**

**Note 1:** Once the Syn-Matrix is placed on the slide, there is only 45 seconds or less to mount the pollen and place the coverslip onto the slide before the medium dries. Work quickly, or unwanted bubbles will form distorting the image of the pollen.

**Note 2:** Bubbles may form for two reasons:

**Reason 1** - The Syn-Matrix has begun to dry before the coverslip is placed. For the best quality, do not wait too long to place the coverslip on the slide, even if working to remove debris. If bubbles form during the drying of the Syn-Matrix, use the non-writing end of a pen to gently press the coverslip down to remove the largest bubbles. Only press on the areas of the coverslip that do not have Syn-Matrix to prevent crushing any pollen grains. Some bubbles may not come out; do not over-press the slides.

**Reason 2** - Ethanol has accompanied the pollen grains onto the Syn-Matrix causing a reaction. To prevent bubbles caused by the ethanol reaction, avoid gathering drops of ethanol from the tubes whenever possible. Allow the sample to sit a few extra seconds when possible before placing the coverslip to allow the ethanol to evaporate. If large amounts of ethanol appear to be clouding the sample, check to ensure that the tool is fully formed and is not drawing in excess ethanol when dipping in the sample. Spreading the Syn-Matrix to areas affected by ethanol-matrix reactions will help lessen the effect.

**Note 3:** While spreading the pollen grains, attempt to place some in polar view, and some in equatorial view so that the species can be fully described at a later time. As previously stated, tapping or pressing too hard on the slide can cause it to move or crush the grains. Be gentle!

1. Hold a clean microscope slide by the edges, and place a small amount of Syn-Matrix in the middle of the slide (see Figure 6). An ideal size would be about 0.5cm in diameter, 0.1cm in height.
2. Place the slide, matrix-side up, under the dissecting microscope. Focus on the drop of Syn-Matrix.
3. Roll the round-tip glass tool in the Syn-Matrix gently to spread the medium slightly and to coat the tool in the medium.
4. Open the lid of the sample and insert the round-tip tool, now coated in Syn-Matrix, carefully into the tube. Gently gather the pollen grains at the bottom of the tube. Do not touch the sides of the tube as you remove the tool.
5. Dab the Syn-Matrix on the microscope slide with the tip of the rounded tool, removing the pollen attached to it. Roll the tool in the Syn-Matrix to remove the pollen grains stuck to the tip.



**Figure 6:** Syn-Matrix drop on slide

6. Use the tool to gently spread out the pollen grains so that they are not clumped. This can be done using a circular motion to draw the grains apart and spread them within the medium. Avoid over-spreading the Syn-matrix to prevent rapid drying and crushing grains during the coverslip mounting process.
7. Remove any unwanted debris from the sample by scooping it out using the needle-tip glass tool, only if time permits.
8. Mount the coverslip to the microscope slide by either gently placing the microscope slide (Syn-Matrix side down) onto the elevated coverslip on the podium, or place the coverslip onto the slide by hand by gently guiding the coverslip over the medium.

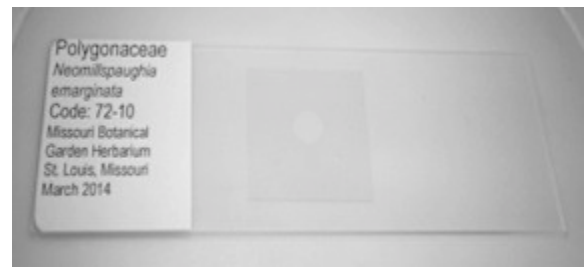
### **Post-Mounting:**

**Note 1:** Ideally, five slides should be mounted for each sample. For samples with low pollen availability, mount as many slides as possible before the sample runs out.

**Note 2:** If pollen is not present in the sample from the start, two slides should be mounted before moving on to the next sample.

### **Slide Labeling and Storage Process**

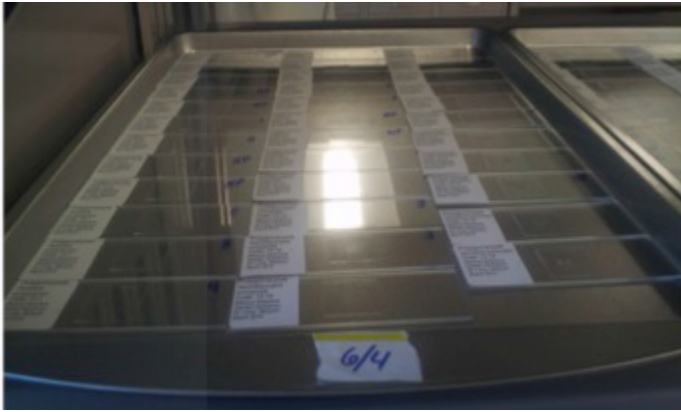
1. Place the label for the sample on the left side of the coverslip (see Figure 7).
2. Observe the slide under a light microscope to determine its quality and amount of pollen. DO NOT view the slide at a magnification greater than 63x for at least two days.
3. Mark the slide in the upper right-hand corner with the following codes, should they apply:
  - a. C - contamination of another species is found on the slide and totals >30% of the pollen present.
  - b. NP - no pollen is present on the slide.
  - c. Number between 1 and 10 - the number of pollen grains present on the slide of the expected species.
  - d. Do not mark the slide if it does not display any of the above characteristics.
4. Use DI water to rinse any glass tools used during the process thoroughly. Wipe the tools completely dry using a Kim wipe, and ensure the tips are clean before moving on to the next sample to prevent contamination. The glass tools are fragile; handle and clean them with care. If a tool breaks, it should be placed in the broken glass disposal container.



**Figure 7:** Labeled slide



5. Place the slides on a level surface to prevent the Syn-Matrix from expanding in one direction (see Figure 8). Slides should not be placed upright, stacked, immersed in oil or observed at 100x magnification for at least two days after mounting to allow the Syn-Matrix to fully dry.
6. Record the date each sample is mounted on the log sheet to prevent accidental movement, description or storage before the Syn-Matrix has fully solidified.
7. Allow the samples to dry for a minimum of 48 hours. The slides can then be moved into the slide storage boxes. They are now ready to be viewed under oil immersion and at 100x magnification (Figure 9).



**Figure 8:** Slides on level tray



**Figure 9:** Slide storage box

# Soil Sampling Protocol

## Materials:

- Hiking boots
- GPS
- Logbook
- Graphite/carbon pencil
- Whirl-pack bags
- Scoopula
- Hiking trail map
- Compass
- Backpack
- Permanent marker



**Figure 1:** Sampling equipment

**Note 1:** Be sure to pack plenty of water, food and sun protection for the trip.

**Note 2:** Hiking boots should be completely clean and sterilized before the start of each collection day.

**Note 3:** Create a hiking itinerary before the collection day. This will provide the collector with detailed information about each site along the collection route. This will also provide a pre-determined starting location.

**Note 4:** Samples should be collected at a minimum distance of 1.61 kilometers (1 mile) from the previous sampling location or when a change of habitat occurs.

## Sample Collection:

1. Record the start of the trip in the GPS and logbook. Provide elevation, time of day, date, area outlook, and coordinates in the logbook.
2. Label the Whirl-pack bag with site location, waypoint number, and sample number.



3. Collect about 1/3 of the Whirl-pack bag by gathering soil from multiple locations within a diameter of 20 meters using the Scoopula (see Figure 2). Ideal samples are moist or wet soils. Avoid sandy soil (see Figure 3).
4. Clean the collection tools thoroughly after each use.



**Figure 2:** Scoopula in use



**Figure 3:** Shows examples of ideal collection sites and a sandy soil site that should not be used

- Place a small label written in pencil with the coordinates and sample number inside of the Whirl-pack bag with the collected sample. The outside of each Whirl-pack bag must also be labeled with a permanent marker, indicating the coordinates and corresponding sample number (see Figure 4). All site information should be saved in the GPS and added to the logbook.

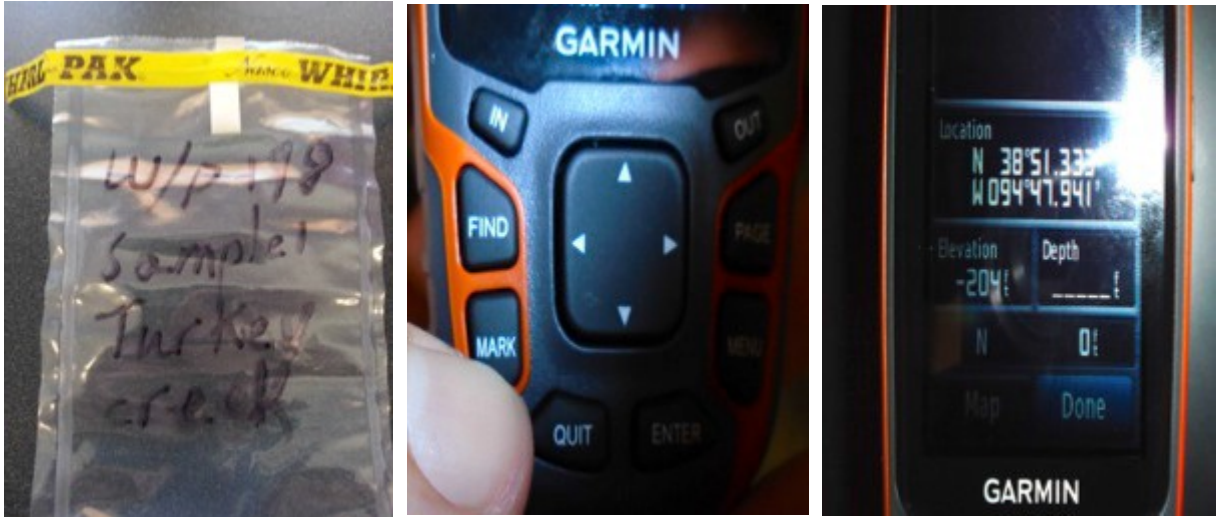


Figure 4: Whirl-pack label example and GPS marking

- Seal the sample properly by extracting as much air as possible from the Whirl-pack bag (see Figure 5). Store the sample in a backpack, and continue to the next sample site.



Figure 5: Sealing the Whirl-pack bag

- Repeat the process using a new Whirl-pack bag at each collection site.
- Scrape the bottom of your boot with the collection tool at the end of the trip to provide a comprehensive control sample.
- See Figure 6 for ideal examples of collection sites.





**Figure 6:** Examples of changes in habitat

# **Soil Sample Preparation Protocol**

(Modified from Faegri and Iversen<sup>1</sup>; Moore and Webb<sup>2</sup> protocols)

## **Materials**

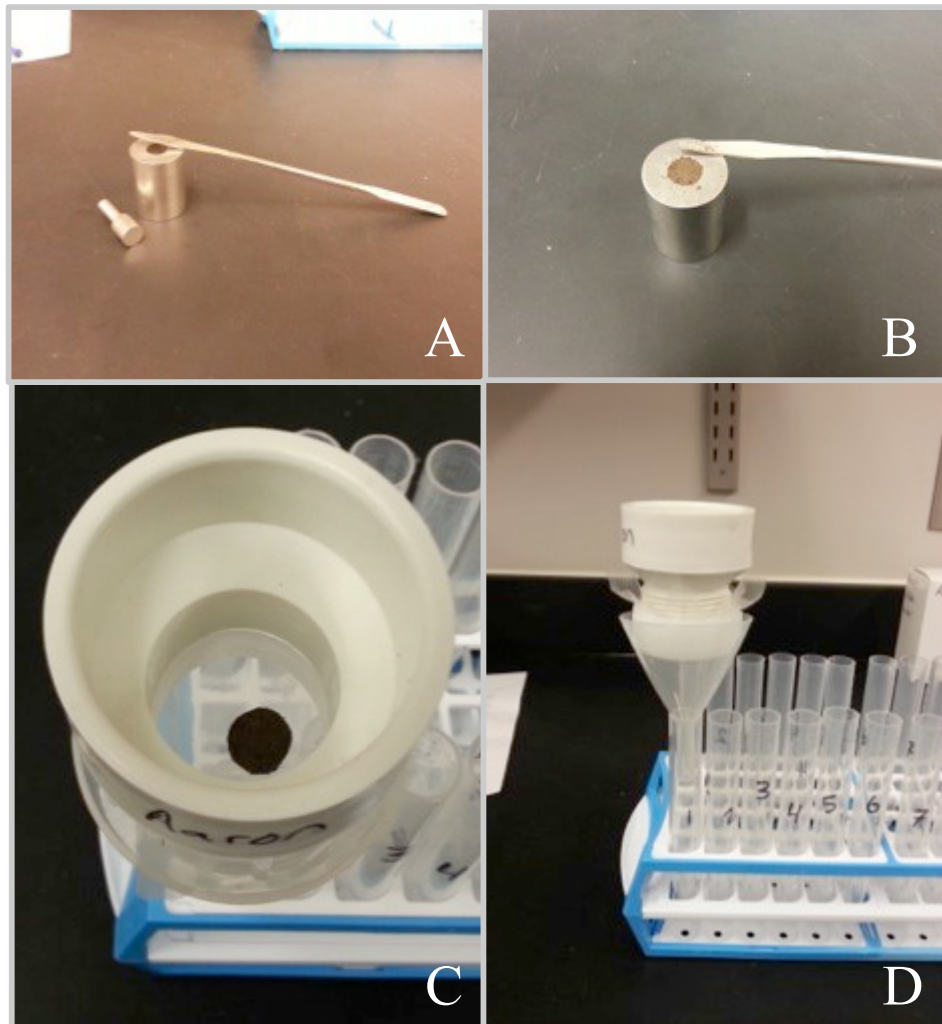
- Sediment samples
- Volumetric sampler
- Metal spatula
- Permanent marker
- 15 mL Conical centrifuge tubes
- 1.7 mL Microcentrifuge tubes
- Test tube rack
- 10% hydrochloric acid solution
- 10% potassium hydroxide solution
- 10% sodium pyrophosphate solution
- Glacial acetic acid
- Concentrated sulfuric acid
- Acetic anhydride
- 25 mL Graduated cylinder
- Sodium metatungstate solution
- Heat block with aluminum element for both 1.7 mL and 15 mL tubes
- Microcentrifuge with inserts for 1.7 mL and 15 mL tubes
- Glass stir rods
- Wooden applicator sticks
- Waste beaker
- 200 µm nylon mesh secured with clamp
- 40 µm nylon mesh
- 2 µm glass filter disc
- Vacuum filtration setup
- Small funnel
- Polystyrene microsphere solution
- 20 µm to 200 µm micropipette
- Glycerol
- Safranin
- Pasteur pipette with bulb
- 10 mL Pipetter
- 10 mL Pipette
- Drying oven
- 1,000 mL Beaker
- 500 mL Amber bottle
- Vortex
- DI Water in wash bottle
- 95% ethanol (if needed)
- Stir plate
- Magnetic stir bar

## **Volumetric Sampling:**

1. Using a small metal spatula and precision volumetric sampler (0.25 cm<sup>3</sup>), remove sediment from the sample bag and press it into the mold of the volumetric sampler until it is level with the rim (see Figure 1A:1B).
2. Remove the 0.25 cm<sup>3</sup> pellet from the sampler and gently place it into a 15 mL polypropylene centrifuge tube.
3. Repeat steps 1 and 2 for each sample bag and location.

**Note:** Samples with large amounts of organic material (greater than 200 µm) will benefit from filtering the samples through a 200 µm screen into the centrifuge tube using deionized water (Figure 1C:1D).

1. Faegri K, Iversen J. 1989. Textbook of pollen analysis 4<sup>th</sup> Ed. Copenhagen: Munksgaard. 237 p.
2. Moore PD, Webb JA, Collinson ME. 1991. Pollen Analysis 2<sup>nd</sup> Edition. Massachusetts: Blackwell Scientific Publications, p 10-28.



**Figure 1:** Equipment for soil sample processing. A: Metal spatula, precision volumetric sampler and plug for 0.25 cm<sup>3</sup> sampling, B: Soil sample in sampler, C: Pellet on mesh screen above centrifuge tube, D: Sample sieving setup composed of 200 µm mesh in PVC collar and funneled to centrifuge tube

### **Microsphere Spiking:**

To quantify the concentration of pollen types present in a sample, 16 µm polystyrene microspheres are added as a marker to the sediment sample at the start of the pollen processing. This allows the pollen concentrations for the soils from different eco-regions to be determined for the geo-location model.

1. Add the determined volume of solution of the microsphere sample to a flask and label it with the concentration.
2. Place a magnetic stir bar into the flask and allow the solution to mix on a stir plate for at least 2 hours (see Figure 2).
3. Use a micropipette to remove 16 µm of microsphere solution from the flask while it continues to stir. Add it to the centrifuge tube.
4. Repeat step 3 for each sample.

**Note:** Traditionally, 20,000 marker spores/cm has been utilized as a marker; therefore, a spike of c. 5000 microspheres/0.25 cm<sup>3</sup> sample should be used. Divide 5,000 by the volume of solution to be added to determine the concentration of the solution (spheres/mL). This ensures that a homogenous solution is maintained and a consistent microsphere spike is added to the sediment sample every time sediment is prepared. If filtering the soil sample, add the microspheres to the surface of the soil pellet and then filter the sample.



**Figure 2:** Microspheres stirring with magnetic rod on stir plate

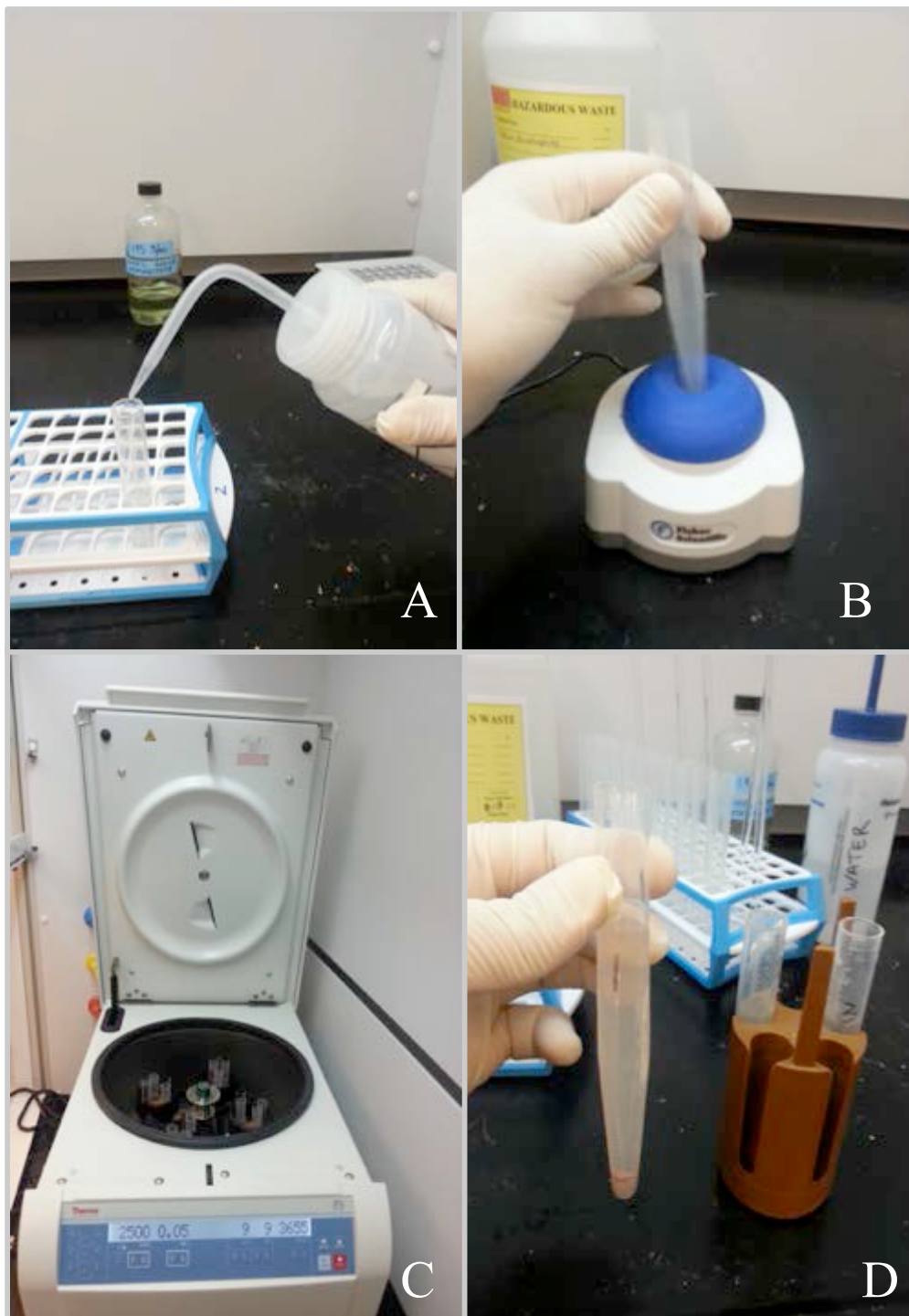
### **Carbonate Removal:**

If the soil is from an area that has carbonate-rich substrate (e.g. limestone, gypsum), an acid wash may be necessary to remove it from the sample. If the sample appears very organic, this step may be skipped.

1. Fill each centrifuge tube to the 5 mL line of the tube with a 10% hydrochloric acid (HCl) solution (Figure 3).
2. Vortex the sample until the soil and acid mix evenly.
3. Place an additional 10% HCl solution into the tube until it reaches the 10 mL line.
4. Place the tubes in a heating block element set on the low temperature setting.
5. Let the samples heat for 10 minutes. If aggressive bubbling or foaming occurs, a second acid wash may need to be conducted to remove the high level of carbonates in the sample.



- Centrifuge the tubes at 2,500 rpm for 5 minutes to pellet the sample after the 10 minute heating period.
- Slowly decant the HCl and dissolved carbonates from the sample pellet.
- Add deionized water to the tubes and vortex for several seconds.
- Centrifuge the samples for 5 minutes at 2,500 rpm to wash the carbonates and HCl from the sample.
- Decant to remove all acid from the sample.



**Figure 3:** Chemical addition or water washing steps. A: Addition of chemical or water, B: Vortexing sample, C: Centrifuging at 2500 rpms for 5 minutes, D: Pelleted sample ready for decanting of solution from sample

## **Tannin and Humic Acid Removal:**

Tannins and humic acids can cause a sediment sample to stick together and darken pollen residue, making it harder to analyze.

1. Fill the centrifuge tubes to the 5 mL line with a 10% potassium hydroxide (KOH) solution and vortex to mix the sample.
2. Continue filling the tubes with 10% KOH to the 10 mL line.
3. Place the tubes in the heat block on the low setting and leave it for 10 minutes. If tannins and humic acids are present in the sample, the solution will become dark brown or black as the compounds are extracted from the sample.
4. Centrifuge the samples at 2,500 rpm for 5 minutes. The sample should appear lighter gray in color if the tannins and humic acids were removed. Samples containing large amounts of organic debris will remain black due to the material, not necessarily the tannins/humic acid.
5. Add DI water to the tubes. Vortex for several seconds and centrifuge for 5 minutes at 2,500 rpm to wash the tannins, humic acid, and KOH from the sample.
6. Decant to remove the base from the sample.

## **Clay Removal:**

Clays are a major component of soils and should be removed to concentrate the sample and prevent clumping.

1. Fill the centrifuge tubes to the 5 mL line with a 10% sodium pyrophosphate solution and vortex until the mixture is homogenous.
2. Continue to fill the tubes to the 10 mL line with a 10% sodium pyrophosphate solution. Then vortex and mix again.
3. Place the tubes in the heat block on the low setting for at least 45 minutes.
4. Centrifuge the tubes for 5 minutes at 2,500 rpm.
5. Slowly decant the solution.
6. Add DI water to the tubes, vortex for several seconds, and centrifuge the tubes for 5 minutes at 2,500 rpm.
7. Slowly decant the solution.

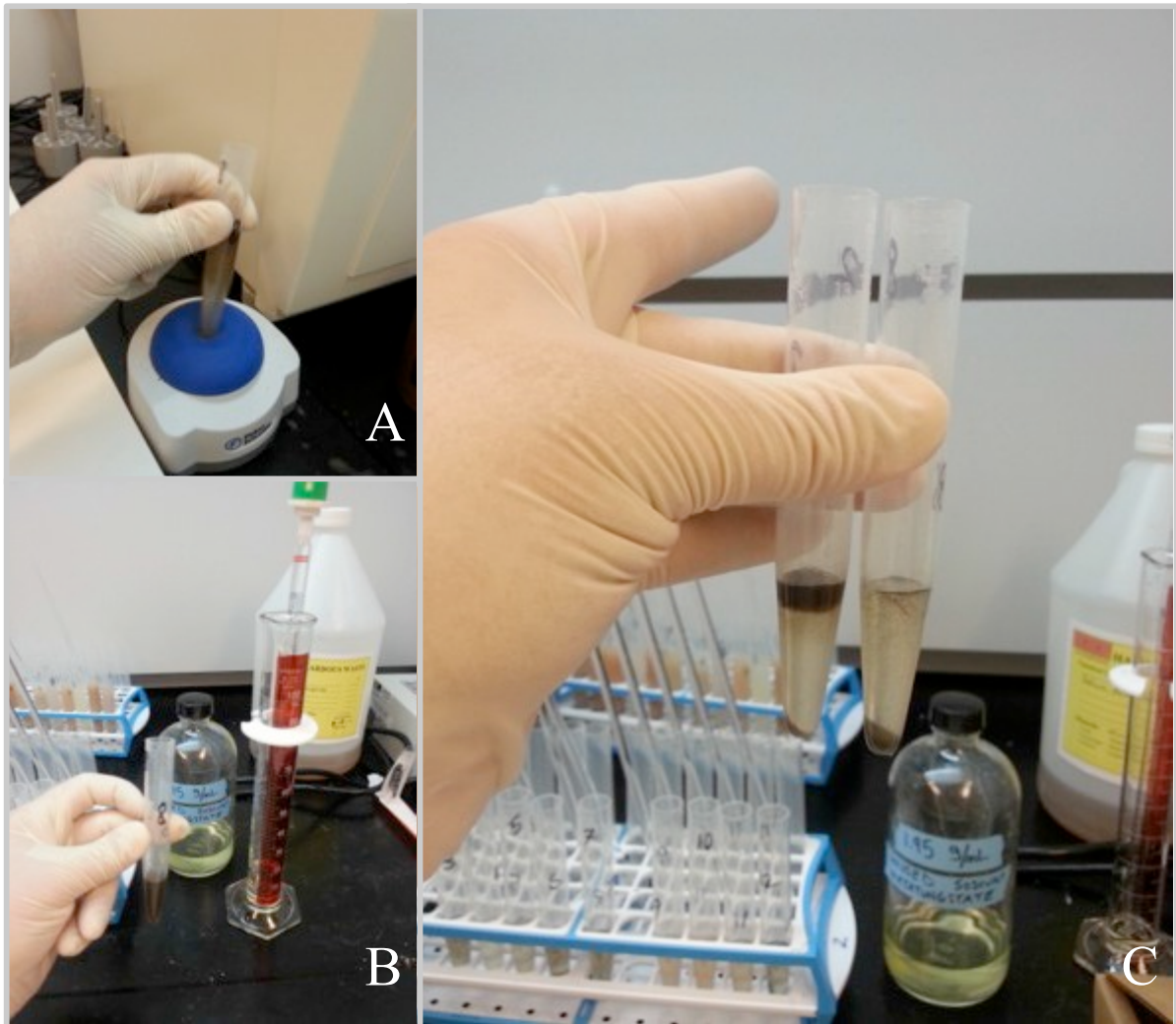
## **Density Separation Using Sodium Metatungstate:**

Concentrate the pollen from denser soil material using a 1.95 g/mL sodium metatungstate solution. If a 1.95 g/mL solution of sodium metatungstate is not available, one should be made from a powdered or concentrated liquid form of the compound. The solution's density is set at 1.95 g/mL in order to allow for the water trapped in the sediment to decrease the sodium metatungstate solution's density, without the solution becoming less dense than the pollen.

1. Add 2 mL of sodium metatungstate solution to each tube and vortex until the sample is completely homogeneous. This may take 3-5 minutes (Figure 4). Large conglomerates of sample will prevent an efficient density separation and allow other soil material into the pollen residue.
2. Allow the solution to settle. The pollen will settle in a layer on top of the soil material.



3. Add 1 mL of sodium metatungstate along the sides of each tube to wash sample off the inner wall of the tube.
4. Centrifuge at no more than 1,800 rpm for 10 minutes. The rpm rate should be increased very slowly over 3-4 minutes to provide the best separation of pollen from other soil components.



**Figure 4:** Steps of density separation with sodium metatungstate - A: Vortexing sodium metatungstate, B: Sample ready for centrifuge, C: Samples after centrifuging with the non-organics pelleted at base of the centrifuge tubes (note differences in amount of organic material to be siphoned from the surface of tube)

5. Siphon the top layer of the solution into a clean centrifuge tube with a Pasteur pipette.
6. Pour the bottom layer (sodium metatungstate and non-pollen soil material) into a beaker for sodium metatungstate reclamation.
7. Add DI water to the new tubes to the 5 mL line and vortex.
8. Add more water to the 10 mL line of the tubes. If needed, add a small squirt of ethanol to break any surface tension.
9. Centrifuge at 2,500 rpm for 5 minutes.
10. Decant the sodium metatungstate solution into the reclamation beaker, leaving the pollen pellet in the tube.
11. Repeat steps 7 through 10 until two washes are completed.

## **Acetolysis:**

Acetolysis solution, composed of a 9:1 ratio of acetic anhydride and concentrated sulfuric acid, will digest all organic material, including pollen grains, so this process should be performed with caution. Soil samples should never be acetolyzed for longer than 5 minutes. The Acetolysis solution will turn the sample a dark yellow or light brown color once the reaction is complete. If there is little or no organic material, the sample may never darken to the yellowish brown color. Do not exceed the 5 minute time frame for the Acetolysis process.

**Caution: The Acetolysis process must be performed in a water-free environment. Be sure to remove any water containers from the fume hood prior to starting this step.**

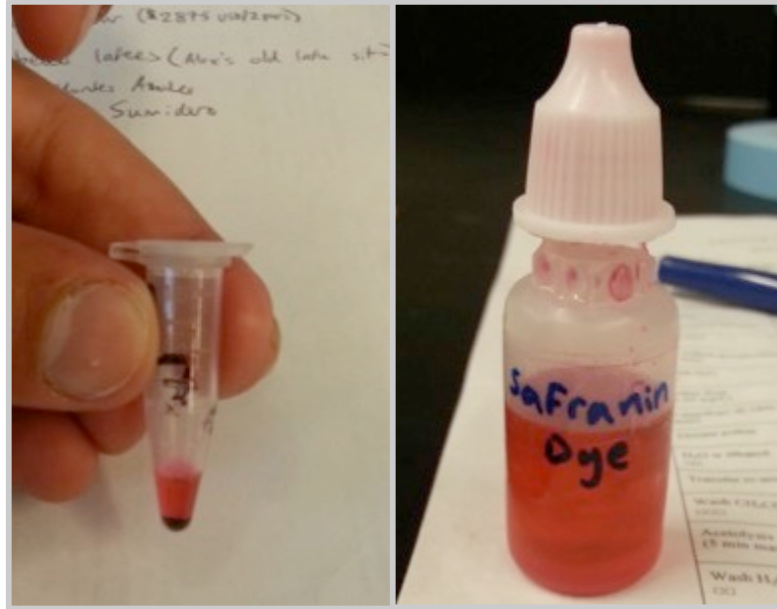
1. Add a very small amount of glacial acetic acid to the sample. Vortex thoroughly and centrifuge at 2,500 rpm. Decant excess glacial acetic acid, being careful to not disturb the sample.
2. Repeat step 1 three times to ensure all water is removed from the sample.
3. Transfer the sample from the centrifuge tube to a 1.7 mL microcentrifuge tube using a Pasteur pipette. Additional glacial acetic acid can be added to the original tube to ensure that the entire sample is transferred.
4. Add 1 mL of Acetolysis solution to each tube.
5. Stir the sample pellet into the Acetolysis solution with a wooden applicator stick.
6. Place the samples in the heat block on the low setting for 5 minutes or less.
7. Remove the tubes from the heat block.
8. Working quickly, add a small amount of glacial acetic acid to each tube to stop the reaction.
9. Centrifuge the tubes at 2,500 rpm for 5 minutes; then decant.
10. Add DI water to the tubes and mix with a wooden stick.
11. Centrifuge the tubes at 2,500 rpm for 5 minutes.
12. Decant to remove all solution from the sample.
13. Repeat this water wash process. If acid can still be detected, complete a third water wash.

## **Adding Fluid Media and Drying the Pollen Residue**

In order to roll pollen grains on the slide during identification, a fluid media (e.g. glycerol) must be used for mounting.

1. Add the same volume of glycerol as volume of the pollen residue to the sample (e.g. 0.25 mL glycerol added to 0.25 mL of residue). Too small of a glycerol addition makes the sample too concentrated and too dark to easily count. Adding too much glycerol will dilute the sample, increasing the counting time for the sample. If a pollen residue is very small, glycerol should be added to the 0.05 mL line of the centrifuge tube so an adequate volume of sample/glycerol mix will be present to make one slide (see Figure 5).

2. Add safranin to the glycerol to stain the pollen surface, allowing for easier identification. A tiny amount (0.05 or 0.06 g) of safranin can be added to a large volume (50-75 mL) of glycerol. The final color of the glycerol should be a shade or two lighter than strawberry jelly. Too dark of a stain will obscure pollen grain surface structure, and too light of a stain will not stain the surface.

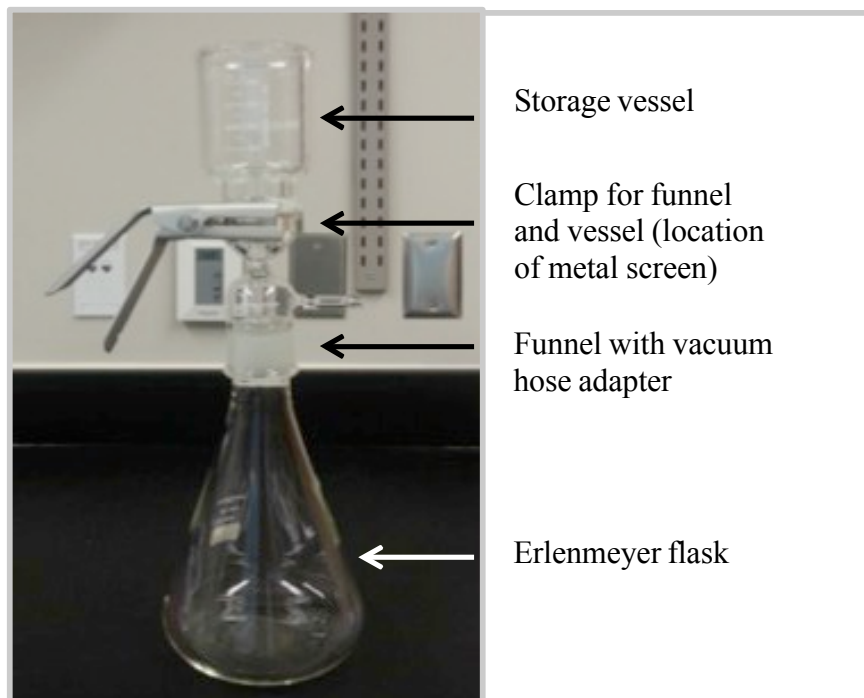


**Figure 5:** Left: Sample filled with glycerol to 0.05 mL level, Right: Glycerol properly tinted with safranin

3. Dry the samples (with caps open) overnight inside an oven (105° C).
4. The following day, smear the sample on slides for counting or store in a refrigerator/cold room for long-term storage.

### **Sodium Metatungstate Reclamation**

Sodium metatungstate is very expensive, so care should be taken to try and recycle the solution after each pollen float procedure. A vacuum filtration set up makes recycling much faster and more effective (Figure 6).



**Figure 6:** Vacuum filtration set up for sodium metatungstate reclamation

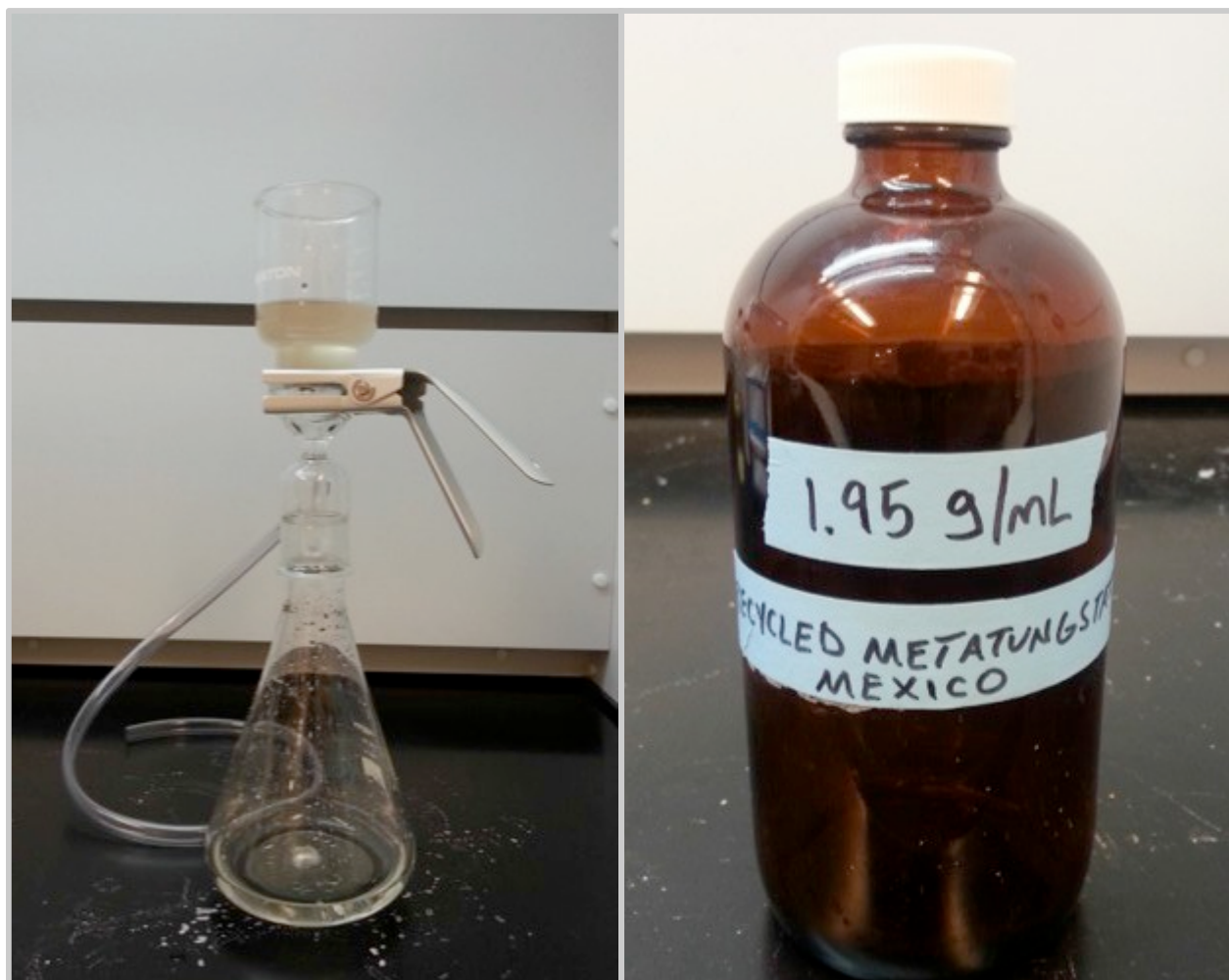
1. Place a 40  $\mu\text{m}$  filter disc on the metal screen that rests on the funnel leading to the Erlenmeyer flask.
2. Clamp the storage vessel onto the funnel and pour the sodium metatungstate reclamation beaker into the vessel.
3. Rinse the beaker into the vessel with DI water.
4. Drain the vessel by gravity and then lightly rinse the sides of the vessel with DI water to remove any sodium metatungstate residue (see Figure 7).



**Figure 7:** Left: Filtration set up, Right top: Metal screen atop funnel and below storage vessel, Right bottom: Large debris caught by 40  $\mu\text{m}$  filter on metal screen



5. Replace the 40  $\mu\text{m}$  filter with a 2  $\mu\text{m}$  filter disc.
6. Pour the filtered sodium metatungstate back into the waste beaker and rinse the Erlenmeyer flask into the beaker.
7. Re-clamp the vacuum filter set up with the 2  $\mu\text{m}$  filter disc on the metal screen.
8. Pour the beaker into the storage vessel and rinse the beaker into the vessel (see Figure 8). The 2  $\mu\text{m}$  filter will require light negative pressure to filter the dirty sodium metatungstate as it moves from the vessel to the Erlenmeyer flask.
9. Connect the hose from the Erlenmeyer flask to the faucet-style mount on the vacuum unit.
10. Slowly open the vacuum unit so that a slow, steady trickle of sodium metatungstate solution moves from the vessel to the flask. Too strong of a vacuum will pull material that should be filtered through the screen.
11. Rinse the beaker into the vessel and rinse the sides of the vessel to remove sodium metatungstate residue.

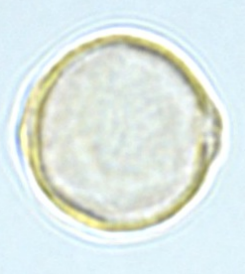
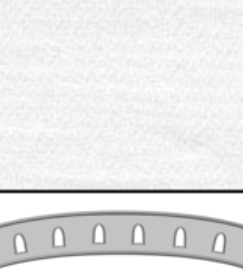
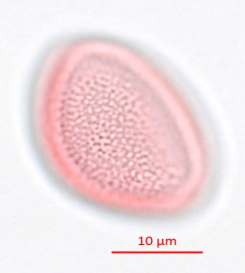
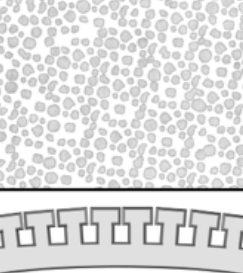

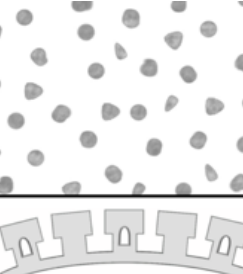

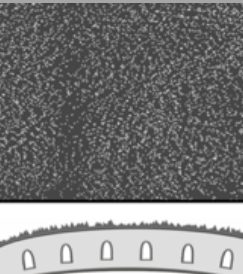

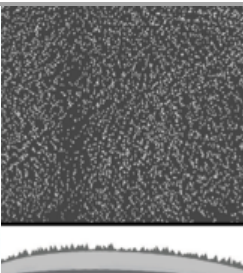
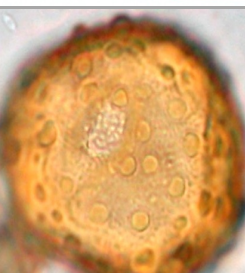
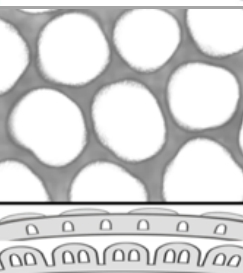



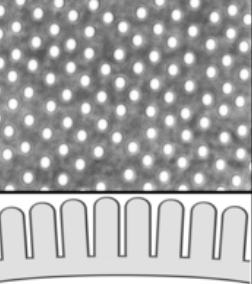

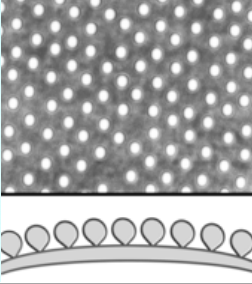

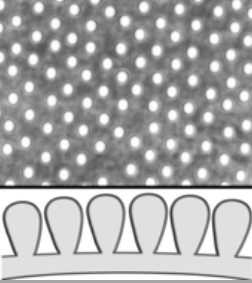
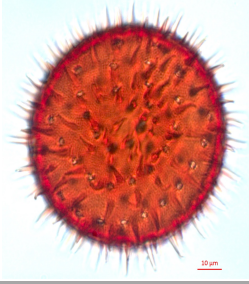
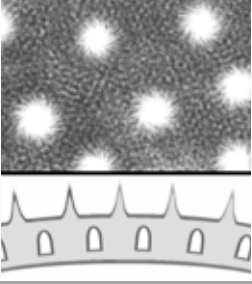

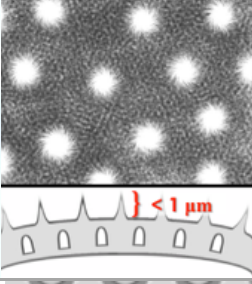
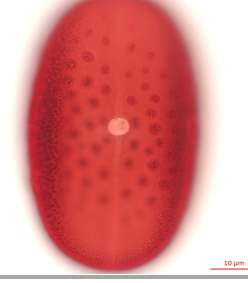
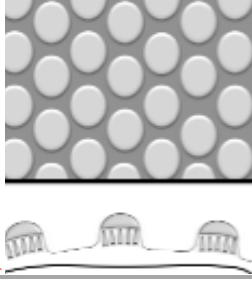
**Figure 8:** Left: Vacuum filtration of fine debris from sodium metatungstate using 2  $\mu\text{m}$  filter and vacuum pump, Right: Properly labeled recycled sodium metatungstate bottle

12. Repeat steps 5 through 11 two to three more times to ensure all contaminating material (pollen and soil particles) is removed.
13. When the 2  $\mu\text{m}$  filter appears clean, filtering is complete.
14. Transfer this solution from the Erlenmeyer flask to the beaker now labeled "clean sodium metatungstate".
15. Rinse the flask into the beaker and concentrate the filtered sodium metatungstate in a drying oven (105° C) over several days.
16. When the metatungstate is denser than 2.5 g/mL, correct the density to 2.00 g/mL and pour from the beaker into a bottle that is labeled "recycled sodium metatungstate" and include the density, as well as sample country of origin (Figure 8).
17. Rinse the beaker with a small amount of water (5 mL).
18. Mix and retest the recycled solution bottle's density. Note any changes on the bottle. As long as the density is between 1.85 and 2.0 g/mL density correction with water or a denser sodium metatungstate solution is not necessary. To prevent cross contamination, each country sampled should have it's own recycled sodium metatungstate solution to use for later soil sample preparations from that country.


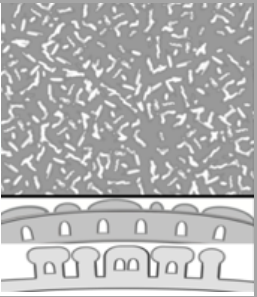
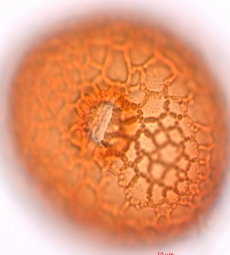
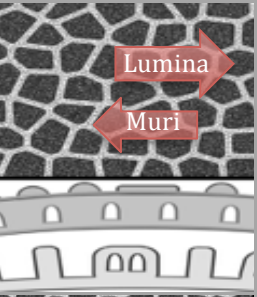

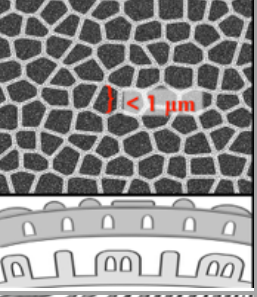

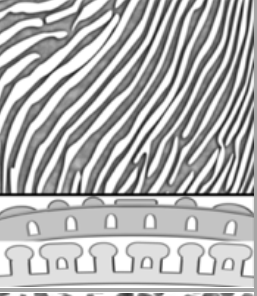

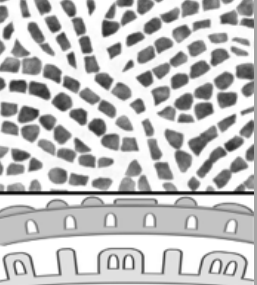
## Pollen Grain Surface Pattern Terminology

*(NOTE: The top panel of the cartoon images below reflects the first plane of focus for the pollen grain. The lighter areas are indicative of protruding structures, such as echini or bacula; the darker areas are indicative of depressions or holes.)*

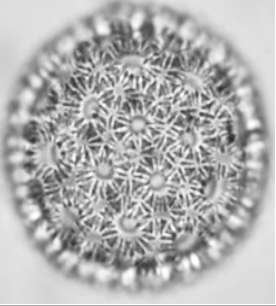
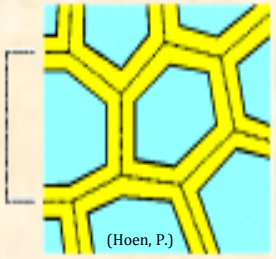
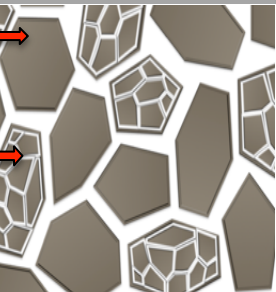
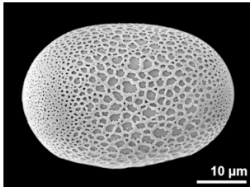
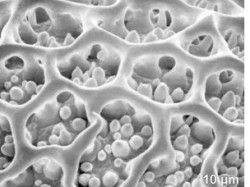
<p>Psilate</p>	<p>Completely smooth surface Exine: Tectate</p> <p style="text-align: right;">Example: Moraceae – <i>Helianthostylis sprucei</i></p>		
<p>Perforate</p>	<p>Surface having small holes or depressions less than 1µm in diameter Exine: Tectate</p> <p style="text-align: right;">Example: Myristicaceae – <i>Virola calophylla</i></p>		
<p>Foveolate</p>	<p>Surface having lumina (holes or depressions) 1µm or greater in diameter; typically the distance between two adjacent lumina is larger than their diameter Exine: Tectate</p> <p style="text-align: right;">Example: Arecaceae – <i>Ammandra decasperma</i></p>		
<p>Scabrate</p>	<p>Any sculptural element less than 1µm in diameter (shape may vary); pattern may appear more irregular (compared to granulate) Exine: Tectate</p> <p style="text-align: right;">Example: Boraginaceae – <i>Hydrophyllum canadense</i></p>		
<p>Granulate</p>	<p>Any sculptural element less than 1µm in diameter (shape may vary) Exine: Intectate</p> <p style="text-align: right;">Example: Anacardiaceae – <i>Rhus</i> sp.</p>		
<p>Verrucate</p>	<p>“Wart-like” sculpturing elements more than 1µm tall, typically broader than they are high; never constricted at the base Exine: Tectate, Semitectate; Intectate</p> <p style="text-align: right;">Example: Fabaceae (C) – <i>Brownneopsis ucayalina</i></p>		

<p>Baculate</p>	<p>Rod-shaped sculpturing elements (bacula), longer than wide and greater than 1 <math>\mu\text{m}</math> high Exine: Intectate</p> <p>Example: Rubiaceae – <i>Galianthe grandifolia</i></p>		
<p>Gemmate</p>	<p>Sculpturing elements (gemma) higher than 1 <math>\mu\text{m}</math>; approximately the same width as height; constricted at their base; “balloon-like” Exine: Intectate</p> <p>Example: Malvaceae – <i>Septotheca tessimannii</i></p>		
<p>Clavate/Pilate</p>	<p>Club-shaped sculpturing elements (clavae), or rods with knob heads, appearing “lollipop-like” (pila); height greater than 1 <math>\mu\text{m}</math>; diameter of clavae or pila is smaller than its height; thicker at apex than at base. Exine: Intectate</p> <p>Example: Euphorbiaceae – <i>Pausandra morisiana</i></p>		
<p>Echinate</p>	<p>Pointed sculpturing elements (echini) 1 <math>\mu\text{m}</math> or greater in height Exine: Tectate</p> <p>Example: Malvaceae – <i>Sidalcea neomexicana</i></p>		
<p>Microechinate</p>	<p>Pointed sculpturing elements (echini) less than 1 <math>\mu\text{m}</math> in height Exine: Tectate</p> <p>Example: Lamiaceae – <i>Aegiphila integrifolia</i></p>		
<p>Areola</p>	<p>Small, and mostly convex, exine islands separated by grooves; a form of “negative reticulum” Exine: Tectate</p> <p>Example: Acanthaceae – <i>Justicia carnea</i></p>		





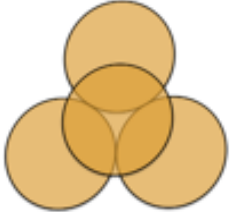
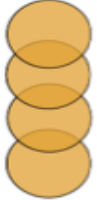
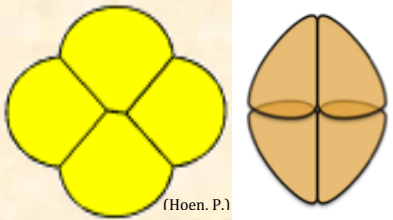
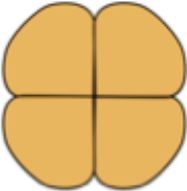
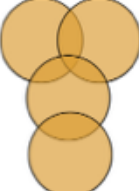
<p>Rugulate</p>	<p>Elongated sculpturing elements greater than 1µm long; pattern irregularly arranged; may resemble an intermediate between reticulate and striate, but this is not necessarily the case</p> <p>Exine: Tectate, Semitectate</p> <p>Example: Violaceae – <i>Rinorea racemosa</i></p>		
<p>Reticulate</p>	<p>Sculpturing elements as ridges arranged in a network which has gaps (lumina) 1µm or greater in diameter; Muri (breadth of ridges) equal to or narrower than the width of the lumina; also described as “network-like pattern” formed by muri</p> <p>Exine: Tectate; Semitectate</p> <p>Example: Malvaceae – <i>Ochroma lagopus</i></p>		
<p>Microreticulate</p>	<p>Appears as reticulate, however, the lumina <u>less than</u> 1µm in diameter</p> <p>Exine: Tectate; Semitectate</p> <p>Example: Acanthaceae – <i>Justicia adhotoda</i></p>		
<p>Striate</p>	<p>Sculpturing elements elongated with the length at least 2 times the width; running more or less parallel; Ridges = muri; gaps between = grooves; surface may look like a fingerprint</p> <p>Exine: Tectate, Semitectate</p> <p>Example: Rosaceae – <i>Prunus reflexa</i></p>		
<p>Striate-reticulate</p>	<p>A pattern in which parallel rows of muri are linked to form reticulum within the grooves; the connections between the muri may lie on a single level or different levels. Exine: Tectate; Semitectate</p> <p>Example: Anacardiaceae – <i>Cyrtocarpa edulis</i></p>		



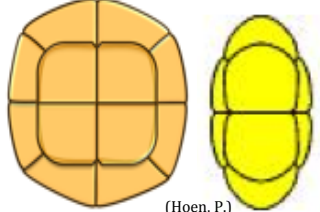
## Reticulate Pollen Terminology

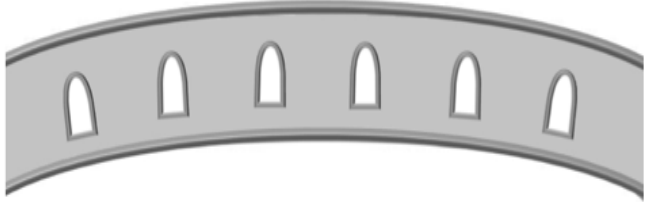
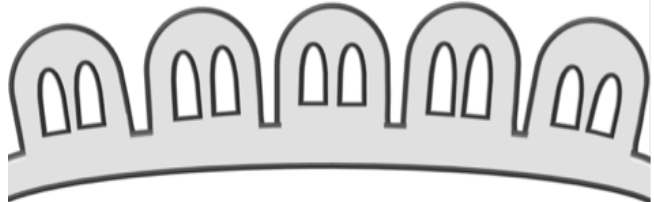
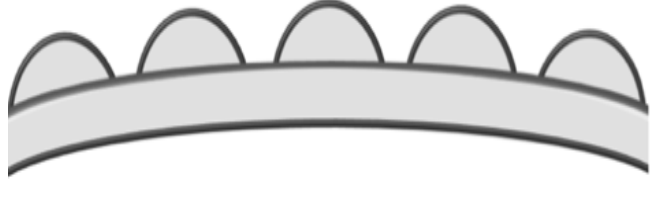
<p>Reticulum Cristatum</p> <p>Croton pattern</p>	<p>Special type of reticulum; Muri has prominent sculpturing elements; add this description to the “Note” section in the database</p> <p>Special type of reticulum cristatum with regularly arranged elements on muri; typically comprised of five to six raised sculpturing elements around a circular area; add this description to the “Note” section in the database</p> <p style="text-align: right;">Example: Buxaceae – <i>Pachysandra procumbens</i></p>	
<p>Brochus</p>	<p>One lumen of a reticulum and half of the width of the surrounding muri</p> <p>Plural: brochi</p>	 <p style="text-align: right;">(Hoen, P.)</p>
<p>Bi-reticulate</p>	<p>Large meshed reticulate (suprareticulum) filled with smaller meshed reticulate (microreticulum); can be categorized under heterobrochate</p>	
<p>Heterobrochate</p>	<p>Reticulated pollen surface with brochi of varying sizes; the size variation may be random or gradual</p>	 <p style="text-align: right;">(Hesse, et al.)</p> <p style="font-size: small;"><i>Dyckia rariflora</i> (Bromeliaceae) 2. polar proximal view Picture credit: Halbritter H.</p>
<p>Homobrochate</p>	<p>Reticulated pollen wall with brochi of uniform size</p>	 <p style="text-align: right;">(Hesse, et al.)</p> <p style="font-size: small;"><i>Dipteracanthus devosianus</i> (Acanthaceae) 4. exine surface Picture credit: Halbritter H.</p>

## Pollen Grain Dispersal Form







*(NOTE: A dispersal unit for pollen is considered as mature or fully developed pollen grains.)*




<p>Monad</p>	<p>Dispersal unit consisting of a single pollen grain</p>	
<p>Dyad</p>	<p>Dispersal unit consisting of two pollen grains</p>	
<p>Tetrad</p>	<p>Dispersal unit of four pollen grains</p>	
<p>Linear Tetrad</p>	<p>Uniplanar tetrad where four units are arranged in a row</p>	
<p>Rhomboidal Tetrad</p>	<p>Uniplanar tetrad with the proximal sides of two individual units in direct contact, and the remaining two units are separated</p>	 <p style="text-align: right; font-size: small;">(Hoen. P.)</p>
<p>Tetragonal Tetrad</p>	<p>Uniplanar tetrad where all four units are in contact at the center for the tetrad forming a cross</p>	
<p>T-Shaped Tetrad</p>	<p>Uniplanar tetrad with two of the units perpendicular to the other two forming a "T" shape</p>	







Tetrahedral Tetrad	Multiplanar tetrad with each unit in contact with the other three units	
Decussate Tetrad	A tetrad of pollen grains arranged in two pairs lying across one another, the pairs (dyads) more or less at right angles to each other	
Polyad	Dispersal unit consisting of <u>more</u> than four pollen grains	 <small>(Hoen. P.)</small>

<b>Pollen Grain Exine Types</b>		
Tectate	Pollen grain with a continuous tectum; also known as 'eutectate'	
Semitectate	Discontinuous tectum that covers less than 50% of the pollen grain's surface	
Intectate	Pollen grain lacking a tectum; also known as 'atectate'	





## Pollen Grain Terminology - Polar Shape

Circular	The length of the vertical axis and horizontal axis are approximately equal; ratio of 1:1	
Elliptic	The length of the vertical axis and horizontal axis are unequal, one having a greater length than the other	
Triangular Convex	A triangular shaped grain with protruding walls connecting two apices	
Triangular Concave	A triangular shaped grain with walls sloping inward connecting two apices	
Triangular Straight	A triangular shaped grain with a relatively straight wall connecting two apices	
Quadrangular	A single unit pollen grain having four angles (degrees may vary) and four sides	
Quinquangular	A single unit pollen grain having five angles (degrees may vary) and five sides	



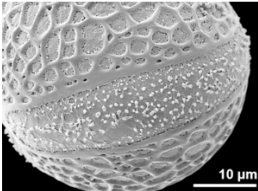
Lobate	A single unit pollen grain having distinctive lobes created by the inward folding of the apertures of the grain			
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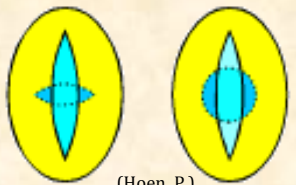
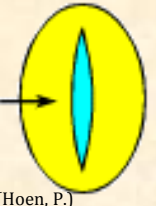
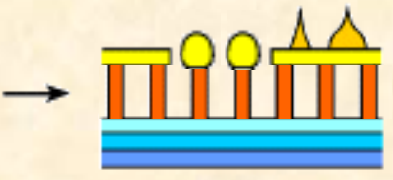
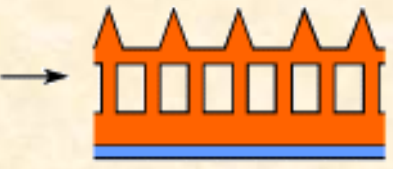
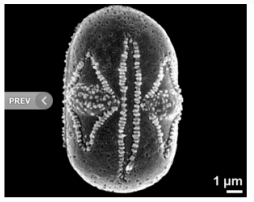
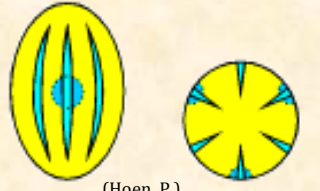

<b>Pollen Grain Terminology - Equitorial Shape</b>		
Oblate	A grain with a polar axis that is shorter than the equatorial diameter in a ratio of approximately 0.5 - 0.75 : 1; (Polar length is 50% to 75% of equatorial length)	
Suboblate	A grain with a polar axis that is shorter than the equatorial diameter in a ratio of approximately 0.75 - 0.95 : 1; (Polar length is 75% to 95% of equatorial length)	
Circular	A grain with a polar axis and equatorial axis that are approximately equal in a ratio of 1 : 1 (Polar and equatorial is relatively equivalent in length)	
Subprolate	A grain with a polar axis that is greater than the equatorial diameter in a ratio of approximately 1 : 0.75 - 0.95 (Equatorial length is 75% to 95% of polar length)	
Prolate	A grain with a polar axis that is greater than the equatorial diameter in a ratio of approximately 1 : 0.5 - 0.75 (Equatorial is 50% to 75% of Polar)	
Perprolate	A grain with a polar axis that is <u>greater</u> than the equatorial diameter in a ratio of approximately 2 : 1 (Equatorial is 50% or less of Polar)	


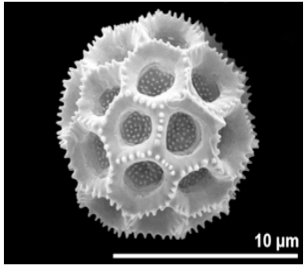
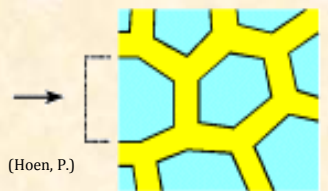

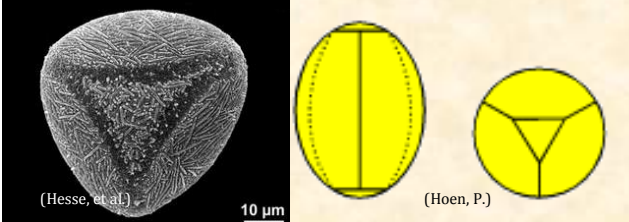
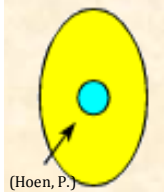




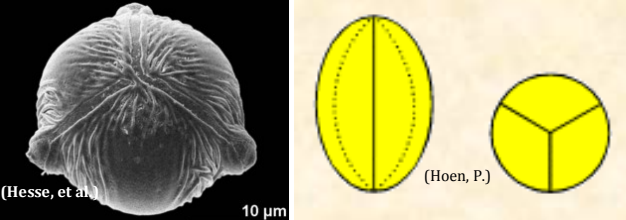

Rectangular Tall	An angular grain with four corner and four sides; polar axis is greater than the equatorial axis	
Rectangular Broad	An angular grain with four corner and four sides; polar axis is less than the equatorial axis	
Rhombic Tall	Oblique-equilaterally shaped grain with four angles and four sides; the polar axis is greater than the equatorial axis	
Rhombic Broad	Oblique-equilaterally shaped grain with four angles and four sides; the equatorial axis is greater than the polar axis	

### Common Pollen Morphology Terminology

Annulus	An area of the exine surrounding a pore that is noticeably differentiated from the remainder of the exine, either in ornamentation or thickness; “ring”	 (Hoen, P.)
Aperture	Region of the pollen wall that is morphologically and/or morphologically different from the rest of the wall; typically thinner than the surrounding wall. Apertures = site of germination. The pollen tube emerges through the aperture, that particular aperture becomes a “germinal” aperture.	 (Hoen, P.)
Aperture Membrane Ornamentation	The exine layer covering the aperture with noticeably distinct ornamentation; typically a different pattern on the aperture than on the surface of the grain	 (Hesse, et al.)  <small><i>Dicentra spectabilis</i> (Papaveraceae) 9. aperture Picture credit: Halbritter H.</small>

Colporus	Compound aperture composed of a colpus and pore; plural: colpori	 <p>(Hoen, P.)</p>
Colpus	Elongated aperture positioned along the equatorial region of the pollen grain or regularly distributed over the grain; plural: colpi	 <p>(Hoen, P.)</p>
Columella	Rod-like structure element often used to support the tectum. Can also be free-standing, as found in semi-tectate grains; plural: columellae;	 <p>(Hoen, P.)</p>
Exine	Outer layer of the pollen wall	 <p>(Hoen, P.)</p>
Heteroaperturate	Pollen grain with two different types of apertures; only one type of aperture is functional, serving as the site of germination; the term typically applies to pollen grains with alternating colpi and Colpori; the term heterocolpate could be used to describe a grain that has two different types of colpi (see definition below)	 <p>1 <math>\mu</math>m</p> <p>(Hesse, et al.)</p> <p><i>Myosotis ramosissima</i> (Boraginaceae) 4, equatorial view Picture credit: Halbritter H.</p>
Heterocolpate	Pollen grains that possess two or more types of colpi, one of which differs in length and/or presence or absence of endoapertures (pori), exine thinning, invaginations or other structures	 <p>(Hoen, P.)</p>
Heteropolar	Grain in which the distal and proximal faces of the exine are different, either in shape, ornamentation or apertural system; the red line in the image divides the proximal and distal faces of the grain, divided at the level of the pore	 <p>(Hoen, P.)</p>

<p>Isopolar</p>	<p>Grain in which the proximal and distal faces of the exine are alike</p>	 <p>(Hoen, P.)</p>
<p>Lacuna</p> <p>Lophae a/k/a "Fenestrate"</p>	<p>Depressed area surrounded by ridges (lophae) in lophate pollen grains; plural: lacunae</p> <p>Window-like pattern of ridges (=lophae) formed by an outer exine surrounding window-like spaces or depressions</p>	 <p>(Hesse, et al.)</p> <p><i>Pfaffia gnaphaloides</i> (Amaranthaceae) 1. hydrated pollen grain Picture credit: Halbritter H.</p>
<p>Lumen</p>	<p>General term for space enclosed by muri (depicted in light blue); plural: lumina</p>	 <p>(Hoen, P.)</p>
<p>Nexine</p>	<p>The inner, non-sculptured part of the exine, which lies below the sexine</p>	 <p>(Hoen, P.)</p>
<p>Parasyncolpate Parasyncolporate</p>	<p>Grain with apertures that are split and attach to the adjacent aperture creating a triangular shape at the pole</p>	 <p>(Hesse, et al.)</p> <p>(Hoen, P.)</p>
<p>Pore</p>	<p>The endoaperture(s) situated at the equator or disbursed evenly over the pollen grain</p>	 <p>(Hoen, P.)</p>
<p>Pseudocolpus</p>	<p>Colpus in heteroaperturate pollen grains, assumed to be a non-functional aperture</p>	 <p>(Hesse, et al.)</p>

Sexine	The sculptured outer layer of the exine	 <p>(Hoen, P.)</p>
Syncolpate Syncolporate	Grain with apertures that have been fused together at one or both poles	 <p>(Hesse, et al.)</p> <p>(Hoen, P.)</p>
Tectum	Outer layer of the exine; can be tectate, semitectate, or intectate	 <p>(Hoen, P.)</p>

Work Cited:

Photographs and Definitions From-

1. Faegri, K., Iversen, J., Kaland, P., & Krzywinski. *Textbook of Pollen Analysis* (IV ed.). Caldwell, New Jersey: The Blackburn Press.
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4. Moore, P., Webb, J., & Collinson, M. (1991). *Pollen Analysis* (Second Edition ed.). Oxford: Blackwell Scientific Publications.

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