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

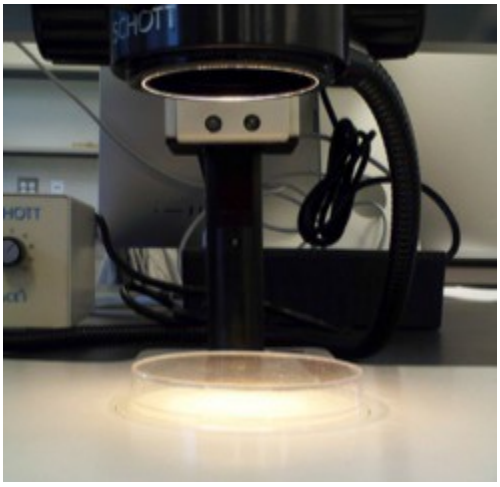

Figure 5: Petri dish on dissecting scope stage

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:

Figure 7: Labeled slide

   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.

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).

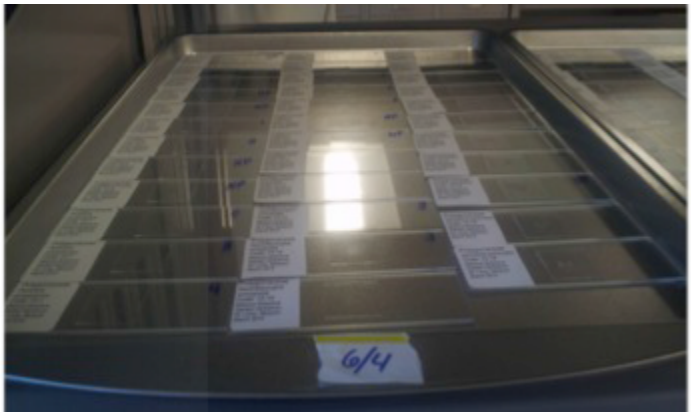

Figure 8: Slides on level tray

Figure 9: Slide storage box