



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L601

Preparing a Wet Mount for Microscopy

Standard Operating Procedure

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1. Purpose

This procedure describes how to prepare a wet mount slide for light microscopy. A wet mount suspends a specimen in a thin layer of water (or other mounting medium) beneath a cover slip, preserving the natural shape and motility of living cells and small organisms. It is the standard preparation method for examining fresh biological samples under bright field or phase contrast illumination (Murphy & Davidson, 2012).

2. Scope

- **Applies to:** Preparation of temporary wet mount slides for examination of algae, protozoa, bacteria, fungi, plant cells, soil microorganisms, and other small specimens in the EA Program laboratory
- **Does not apply to:** Permanent slide preparation (fixed, stained, and sealed mounts); dry mounts; hanging-drop preparations for motility studies; fluorescence specimen preparation (see [SOP-L211](#))
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Trained Personnel	Prepare slides following this procedure
New Students	Complete training before independent use

4. Definitions

- **Cover slip (coverslip):** A thin (0.13–0.17 mm) square or circular piece of optical glass placed over the specimen on a microscope slide; standardizes the optical path length and protects the objective from liquid samples; the EA Program standard is No. 1.5 (0.17 mm), matched to the objectives on the Echo Revolve microscope (Murphy & Davidson, 2012).
- **Immersion medium:** Liquid placed between the specimen and the coverslip to improve optical contact, reduce refraction artifacts, and support live organisms during observation; options include water, glycerol, or immersion oil depending on the application.
- **Live mount:** A wet mount prepared without fixation or staining; allows observation of living organisms and motile behavior; must be examined quickly before evaporation or oxygen depletion affects the specimen.
- **Mounting medium:** The liquid in which the specimen is suspended; distilled or deionized water is standard; saline, glycerol, or specific staining solutions may be used depending on the application (Murphy & Davidson, 2012).
- **Refraction artifact:** Optical distortion caused by differences in refractive index between the sample, mounting medium, coverslip, and objective; minimized by matching refractive indices and maintaining thin, uniform wet mounts.

- **Wet mount:** A temporary microscopy preparation method in which a specimen is suspended in a drop of liquid (usually water) on a glass slide and covered with a coverslip; the standard preparation for aquatic microorganisms, algae, macroinvertebrates, and other specimens examined under transmitted light (Murphy & Davidson, 2012).

5. Safety and Hazards

Hazards

- **Physical:** Microscope slides and cover slips are fragile glass. Broken glass can cause cuts. Handle by the edges and dispose of chipped or cracked glass in the broken-glass box.
- **Biological:** Environmental samples (water, soil, compost) may contain unknown microorganisms. Treat all samples as potentially infectious and wash hands after handling.

5.1. Required PPE

- ☐ Safety glasses
- ☐ Lab coat
- ☐ Nitrile gloves

5.2. Emergency Procedures

For cuts from broken glass, apply direct pressure and rinse with water. Seek first aid if bleeding is significant. Refer to [SOP-L002 Laboratory Safety](#).

6. Required Materials

Item	Catalog No.	Notes
Microscope slides (75 × 25 mm)	TBD	Standard glass, pre-cleaned
Cover slips (No. 1.5, 22 × 22 mm)	TBD	0.17 mm thickness; matched to objectives on the Revolve microscope (Murphy & Davidson, 2012)
Squeeze bottle with DI water		Or distilled water
Disposable transfer pipettes or eyedroppers		For placing water drops
Forceps (fine-tipped)		For handling specimens and cover slips
Toothpicks		For lowering cover slips
Lint-free wipes (Kimwipes)		For blotting excess water

i Catalog Numbers Needed

The original SOP flagged that catalog numbers for slides and cover slips should be added. Update this table when part numbers are confirmed with the lab manager.

7. Estimated Time

This procedure requires approximately 5 minutes per slide.

8. Procedure

8.1. Placing the Specimen

1. Clean a microscope slide with a lint-free wipe to remove dust and fingerprints.
2. Using forceps, place the specimen in the center of the slide. For liquid samples (pond water, culture broth), skip to Step 3 and pipette the sample directly.

8.2. Adding the Mounting Liquid

3. Using a transfer pipette or eyedropper, place one to two drops of water onto the specimen. Adjust the volume based on specimen size: larger or thicker specimens may need slightly more water, while very small specimens need less.

8.3. Applying the Cover Slip

4. Pick up a cover slip by its edges with forceps or clean, dry fingers. Never touch the flat surfaces.
5. Place one edge of the cover slip on the slide adjacent to the specimen, holding it at approximately a 45-degree angle.
6. Slowly lower the cover slip using the tip of a toothpick or forceps, allowing the water to spread gradually beneath the glass. Lowering at an angle lets air escape and minimizes bubble formation (Murphy & Davidson, 2012).

💡 Avoiding Air Bubbles

Air bubbles distort the image and can be mistaken for cellular structures. If bubbles are present after placing the cover slip, gently tap the cover slip with the eraser end of a pencil or press lightly with a toothpick to work bubbles toward the edge. If large bubbles persist, remove the cover slip, add a fresh drop of water, and try again.

8.4. Adjusting Water Volume

7. **Too much water:** If the cover slip floats or slides freely, the water layer is too thick. Hold the edge of a lint-free wipe against the edge of the cover slip and let capillary action draw off excess water until the cover slip sits flat.
8. **Too little water:** If dry areas are visible beneath the cover slip, place a small drop of water at the edge and let capillary action draw it underneath. Do not lift the cover slip.

8.5. Examining the Slide

9. Place the prepared slide on the microscope stage and examine following [SOP-L211 Echo Labs Revolve Microscope](#). Start at low magnification (4× or 10×) and increase as needed.
10. For unstained, transparent specimens, phase contrast mode typically provides better visibility than bright field (see SOP-L211, Section 6.5).

9. Quality Control

- Verify that the cover slip lies flat and is not tilted by air bubbles or excess sample material.
- Confirm that the water layer is thin enough for the specimen to remain in a single focal plane at high magnification.
- If examining motile organisms, note that excessive pressure from the cover slip can restrict movement; use slightly more water to provide adequate space.

10. Waste Disposal

Waste Type	Disposal Method
Used glass slides and cover slips	Broken-glass box
Used toothpicks and wipes	Standard trash
Biological specimen waste	Biohazard bag if potentially infectious; otherwise standard trash

11. Troubleshooting

Problem	Probable Cause	Corrective Action
Air bubbles under cover slip	Cover slip lowered too quickly	Remove cover slip, re-wet, lower at 45° slowly
Cover slip slides around	Too much water	Blot excess with lint-free wipe
Dry patches under cover slip	Too little water	Add a drop at the edge; capillary action fills the gap
Specimen drifts out of view	Water current under cover slip	Wait for flow to stop; blot if excess water is present

Problem	Probable Cause	Corrective Action
Image blurry at high magnification	Thick water layer or dirty slide	Blot excess water; clean slide and re-prepare

12. References

- Murphy & Davidson (2012)

Murphy, D. B., & Davidson, M. W. (2012). *Fundamentals of light microscopy and electronic imaging* (2nd ed.). Wiley. <https://doi.org/10.1002/9781118382905>

13. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required safety training
L211	Revolve Microscope	Instrument used to examine slides
L602	Macroinvertebrate Sorting	Uses wet mounts for ID
L604	Algae Identification	Uses wet mounts for ID

14. Training Record

Before preparing wet mount slides independently, personnel must:

1. Read this SOP in full
2. Complete [SOP-L002 Laboratory Safety](#) training
3. Complete [SOP-L211 Revolve Microscope](#) training
4. Prepare at least two wet mount slides under supervision and demonstrate proper cover slip technique (no air bubbles, correct water volume)
5. Be signed off by the PI or lab manager