



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L216

Dissecting and Compound Microscope

Standard Operating Procedure

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1. Purpose

This procedure describes the operation, image capture, and routine care of the dissecting (stereo) microscopes and compound light microscopes used in the EA Program laboratory. Dissecting microscopes provide low-to-moderate magnification (typically 7–45x) with a wide working distance and are used for sorting biological specimens, gross morphology, and sample preparation. Compound microscopes provide higher magnification (40–1000x) with transmitted illumination and are used for slide-mounted specimens requiring cellular or subcellular resolution (Murphy & Davidson, 2012).

For the Echo Labs Revolve hybrid microscope (which combines upright compound and inverted configurations with an iPad display), see [SOP-L211 Revolve Microscope](#). The Revolve has specialized procedures that differ from the conventional microscopes described here.

2. Scope

- **Applies to:** All conventional dissecting (stereo) microscopes and compound light microscopes in the EA Program laboratory, including units with and without attached cameras
- **Does not apply to:** The Echo Labs Revolve microscope (see [SOP-L211](#)); electron microscopy; confocal microscopy; fluorescence microscopy on non-Revolve instruments
- **Location:** EA Program laboratory, SGM 131/133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, authorize instrument access
Lab Manager	Schedule maintenance, manage optics cleaning supplies, maintain camera software
Trained Personnel	Follow this SOP, report malfunctions
New Students	Complete training before independent use

4. Definitions

- **Brightfield microscopy:** Transmitted-light microscopy in which the specimen is illuminated from below and appears against a bright background; the standard mode for stained biological specimens.
- **Compound microscope:** A microscope with two or more lens systems (objective and eyepiece) that produce a magnified image; used for cells, microorganisms, and fine structural detail at total magnifications of 40x–1,000x.
- **Condenser:** A lens assembly below the stage that focuses and concentrates the illumination light onto the specimen; its numerical aperture and position strongly affect image contrast and resolution.
- **Dissecting microscope (stereo microscope):** A low-magnification (typically 7x–45x) binocular microscope that provides a three-dimensional image of intact specimens; used for macroinvertebrate sorting, plant tissue examination, and dissection.
- **Immersion oil:** A synthetic or natural oil with a refractive index matching glass (~1.515); applied between the 100x objective and the coverslip to eliminate the air-glass interface and maximize numerical aperture.
- **Iris diaphragm (condenser diaphragm):** An adjustable aperture in the condenser that controls the cone of illumination; reducing the diaphragm increases contrast but reduces resolution.
- **Parfocal:** The property of a set of objectives that allows switching between magnifications with only minor focus adjustment; standard on modern compound microscopes.
- **Working distance:** The distance between the front lens of the objective and the specimen surface when in focus; decreases with increasing magnification; limits the thickness of specimens that can be accommodated.

5. Safety and Hazards

Hazards

- **Optical:** Prolonged viewing through the eyepieces can cause eye strain. Take breaks during extended sorting or identification sessions. Adjust the diopter rings to match your vision before starting work.
- **Electrical:** Keep liquids away from the illumination base and power supply. If liquid enters the base, disconnect power immediately and notify the lab manager.
- **Physical:** Compound microscope objectives (especially 40x and 100x) can contact and crack the slide or cover slip if the stage is raised too quickly. Always use the coarse focus knob with caution when changing objectives.
- **Chemical/Biological:** Specimens may be preserved in ethanol, formalin, or other fixatives. Wear appropriate PPE and handle preserved material in a well-ventilated area.

5.1. Required PPE

- ☐ Safety glasses (when not viewing through eyepieces)

- ☐ Lab coat
- ☐ Nitrile gloves (when handling preserved specimens, stains, or immersion oil)

5.2. Emergency Procedures

For chemical spills (e.g., ethanol, formalin) on or near a microscope, clean the spill per [SOP-L002 Laboratory Safety](#) and [SOP-L003 Hazardous Materials Handling](#). If liquid enters the microscope body, unplug power and contact the lab manager before attempting further use.

6. Equipment and Materials

6.1. Dissecting (Stereo) Microscopes

Item	Qty	Location	Notes
Dissecting scope with illuminated base	7	SGM 131/133	7–45x zoom; incident and transmitted light
Dissecting scope with camera	1	SGM 133	Camera-equipped for image capture

6.2. Compound Microscopes

Item	Qty	Location	Notes
Compound light microscope	<i>TBD</i>	SGM 133	Objectives: 4x, 10x, 40x, 100x (oil)

6.3. Consumables

Item	Notes
Microscope slides (25 x 75 mm)	Standard glass
Cover slips (No. 1.5, 0.17 mm)	For compound microscope use
Immersion oil (Type A or NF)	For 100x oil immersion objective only
Lens cleaning solution	Optical-grade, for objectives and oculars
Lens cleaning tissue (lint-free)	Use optical-grade tissue, not Kimwipes on coated optics
Compressed gas duster	For removing dust; use short bursts only
Depression slides	For wet mounts of live specimens

7. Procedure

7.1. Choosing the Right Microscope

Task	Microscope Type	Typical Magnification
Sorting macroinvertebrates from debris	Dissecting	7–20x
Gross specimen identification	Dissecting	10–45x
Examining head capsules, chaetae, gill structures	Compound	100–400x
Counting algae or diatoms	Compound	200–400x
Identifying microplastics on filters	Dissecting	10–45x
Viewing stained tissue sections	Compound	40–1000x

7.2. Dissecting Microscope: Setup and Use

1. Plug in the microscope and turn on the illumination using the switch on the base. Most units have both **incident** (top) and **transmitted** (bottom) light sources. Select the mode appropriate for the specimen:
 - Incident light: opaque specimens (insects, soil, rocks)
 - Transmitted light: translucent specimens (small organisms in water, thin sections)
 - Both: general sorting when mixed materials are present
2. Adjust the zoom to the lowest magnification (widest field of view) to locate the area of interest
3. Place the specimen on the stage plate. Use a white plate for dark specimens and a black plate for light or translucent specimens to improve contrast.
4. Adjust the interpupillary distance by sliding the eyepiece tubes until you see a single merged circle of light
5. Focus using the focus knob (single knob on most stereo scopes). With both eyes open, adjust the diopter ring on one eyepiece until the image is sharp in both eyes simultaneously.
6. Increase magnification by turning the zoom dial. Refine focus as needed.
7. When finished, return the zoom to the lowest setting, remove the specimen, turn off the illumination, and unplug the unit if lab policy requires it

7.3. Compound Microscope: Setup and Use

1. Plug in the microscope and turn on the light source using the power switch. Adjust the intensity to a comfortable level.
2. Rotate the nosepiece to the lowest-magnification objective (4x)
3. Place the prepared slide on the stage, securing it with the stage clips. Center the specimen over the condenser aperture.
4. Using the **coarse focus knob**, raise the stage until the specimen comes into approximate focus. Watch from the side to avoid driving the slide into the objective.
5. Sharpen the image with the **fine focus knob**
6. Adjust the condenser height and the iris diaphragm to optimize contrast and resolution. For most work, the condenser should be nearly fully raised (close to the slide) and the diaphragm partially closed to improve contrast without sacrificing too much resolution (Murphy & Davidson, 2012).
7. Increase magnification by rotating the nosepiece to the next objective (10x, then 40x). Refocus with the fine focus knob only.

! Avoid Slide Damage

When switching from the 10x to the 40x objective, watch from the side to confirm that the longer 40x lens does not contact the cover slip. If the working distance is too short, lower the stage slightly before rotating the objective into position.

7.4. Using Oil Immersion (100x Objective)

1. Focus the specimen at 40x first
2. Rotate the nosepiece to an empty position (no objective in the light path)
3. Place a single small drop of immersion oil directly on the cover slip at the point of illumination. Do not use excess oil.
4. Rotate the 100x objective into position. The front element of the lens should contact the oil without trapping an air bubble.
5. Focus carefully using the **fine focus knob only**
6. After use, immediately clean the 100x objective with lens cleaning solution and lint-free tissue. Wipe gently from center to edge. Never allow oil to dry on the lens.
7. Clean the slide or dispose of it as appropriate

7.5. Capturing Images (Camera-Equipped Units)

1. Focus the specimen and center the region of interest in the field of view
2. Open the camera software on the connected computer (software varies by camera model; consult the lab manager for the current setup)
3. Adjust exposure and white balance in the software to match the illumination
4. Capture the image. Record the magnification, scale bar, and sample ID in the file name or metadata.
5. Use a consistent file-naming convention: YYYY-MM-DD_SampleID_Magnification_Mode.jpg
6. Export images to the project data folder

7.6. Shutdown and Daily Maintenance

1. Remove the specimen from the stage
2. Rotate the nosepiece to the lowest-magnification objective (4x on compound; lowest zoom on dissecting)
3. If oil immersion was used, clean the 100x objective immediately (see Section 6.4, step 6)
4. Wipe any residue from the stage with a lint-free tissue
5. Turn off the light source and unplug if required by lab policy
6. Cover the microscope with a dust cover

8. Quality Control and Acceptance Criteria

8.1. Optics Cleaning

If specks or smudges appear in the field of view that do not move when the specimen is translated, the optics need cleaning:

1. Start with a few short bursts of compressed gas duster to remove loose dust from the objectives and oculars. Do not invert the can.
2. For objective lens surfaces, apply a small amount of lens cleaning solution to a piece of lint-free optical tissue and gently wipe in a circular motion from center to edge. Do not use dry tissue on coated optics.
3. Clean the oculars (eyepieces) the same way. Fingerprints on the eye lens are the most common source of image degradation.
4. For persistent contamination on internal optics, contact the lab manager. Do not disassemble the microscope.

8.2. Illumination Check

- Replace burned-out bulbs promptly. LED-equipped units should not require bulb replacement during normal use.
- If transmitted light appears uneven, check that the condenser is properly centered and the iris diaphragm is functioning.

8.3. Image Quality Checklist

Before accepting a micrograph for publication or analysis, verify:

- Focus is sharp across the region of interest
- Illumination is even (no hot spots or vignetting)
- A scale bar or magnification is recorded
- The specimen is oriented consistently with project conventions

9. Waste Disposal

Waste Type	Disposal Method
Used immersion oil tissues	Standard trash
Used microscope slides (non-hazardous)	Broken-glass box if glass
Biological specimen waste	Biohazard bag if applicable; autoclave before disposal per SOP-L112
Preserved specimen waste (ethanol)	Flammable waste container
Preserved specimen waste (formalin)	Hazardous waste; dispose through EH&S
Spent lens cleaning solution	Standard trash (small volumes on tissues)

10. References

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

11. Training Record

Before using dissecting or compound microscopes independently, personnel must:

1. Read this SOP in full
2. Complete [SOP-L002 Laboratory Safety](#) training
3. Demonstrate proper setup, focusing, magnification changes, and shutdown of both dissecting and compound microscopes under supervision
4. If oil immersion work is required, demonstrate proper oil application, focusing, and cleanup
5. If image capture is required, demonstrate camera operation and correct file naming
6. Be signed off by the PI or lab manager

12. Appendix A: Microscope Anatomy Quick Reference

12.1. Dissecting (Stereo) Microscope

Component	Function
Eyepieces (oculars)	Binocular viewing; 10x magnification (typical)
Zoom dial	Continuously adjustable magnification (e.g., 0.7–4.5x objective)
Focus knob	Single knob for coarse/fine focus
Stage plate	Removable; white and black plates for contrast
Incident light	Top illumination for opaque specimens
Transmitted light	Bottom illumination for translucent specimens
Diopter adjustment	On one or both eyepieces; corrects for vision differences between eyes

12.2. Compound Microscope

Component	Function
Eyepieces (oculars)	Binocular viewing; 10x magnification (typical)
Nosepiece (turret)	Holds and rotates objective lenses
Objective lenses	4x, 10x, 40x, 100x (oil); determine magnification
Stage	Holds slide; translatable via X/Y controls
Condenser	Focuses transmitted light onto the specimen
Iris diaphragm	Controls cone angle of illumination; affects contrast and resolution

Component	Function
Coarse focus knob	Rapid focus (large movements)
Fine focus knob	Precise focus (small movements)
Light intensity control	Adjusts brightness of the transmitted light source

13. Appendix B: Total Magnification Reference

Total magnification = eyepiece magnification $\times$ objective magnification. For standard 10x eyepieces:

Objective	Total Magnification	Typical Use
4x	40x	Scanning; locating regions of interest
10x	100x	General observation
40x	400x	Detailed cellular structure
100x (oil)	1000x	Fine detail; bacteria, diatom striae, chromosomes

14. Appendix C: Relationship to Other SOPs

SOP	Relationship
SOP-L211	Echo Labs Revolve hybrid microscope (upright/inverted, iPad display, fluorescence)
SOP-L602	Macroinvertebrate sorting and identification (uses dissecting and compound scopes)
SOP-L308	Microplastics enumeration (uses dissecting scope and Revolve)
SOP-L112	Autoclave / sterilization for biohazard waste