



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L211

Echo Labs Revolve Microscope

Standard Operating Procedure

SOP Number	SOP-L211
Revision	0.1
Author(s)	Dmaia Curry, Aparna Chintapalli
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the operation, image capture, and maintenance of the Echo Labs Revolve microscope. The Revolve is a hybrid instrument that combines an upright microscope (for prepared glass slides) and an inverted microscope (for live samples in dishes) in a single rotating platform, with an iPad retina display replacing the traditional eyepiece (Echo Labs, 2016; Murphy & Davidson, 2012). The microscope supports bright field, phase contrast, fluorescence, and dark field imaging modes. It represents a substantial investment by the EA Program and requires careful handling.

2. Scope

- **Applies to:** All use of the Echo Labs Revolve microscope in the EA Program laboratory, including bright field, phase contrast, fluorescence, and dark field modes in both upright and inverted configurations
- **Does not apply to:** Electron microscopy, confocal microscopy, dissecting/stereo microscopes, or other optical microscopes not described here
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, authorize instrument access
Lab Manager	Schedule maintenance, manage software updates, maintain optics
Trained Personnel	Follow this SOP, report malfunctions
New Students	Complete training (including video modules) before independent use

4. Definitions

- **Brightfield illumination:** Standard transmitted-light microscopy in which the sample is illuminated from below and absorbing or scattering structures appear dark against a bright background; the most common mode for stained biological specimens.

- **Darkfield illumination:** A transmitted-light mode in which the direct beam is blocked; only obliquely scattered light reaches the objective, making unstained transparent structures appear bright against a dark background.
- **Epifluorescence:** A fluorescence microscopy mode in which excitation light is directed through the objective onto the sample, and the emitted fluorescence is collected back through the same objective; enables fluorescent stain visualization.
- **Eyepiece (ocular):** The lens assembly through which the observer views the magnified image; typically 10x; total magnification equals eyepiece magnification times objective magnification.
- **Fluorescence filter cube:** An interchangeable optical block containing an excitation filter, dichroic mirror, and emission filter matched to a specific fluorophore (e.g., DAPI, FITC, TRITC).
- **Numerical aperture (NA):** A dimensionless number characterizing the angular range of light an objective can accept; higher NA provides better resolution and light-gathering; the resolving power is approximately $d = 0.61\lambda/NA$.
- **Objective:** The primary lens closest to the specimen; available in magnifications of 4x, 10x, 20x, 40x, and 100x (oil immersion); higher magnification objectives have shorter working distances.
- **Working distance:** The distance between the front lens of the objective and the coverslip when the specimen is in focus; decreases with increasing magnification; critical for thick samples.

5. Safety and Hazards

Hazards

- **Optical:** The fluorescence LED produces intense illumination. Avoid looking directly into the excitation light source. The iPad display eliminates the need to view through an eyepiece, reducing direct optical exposure, but reflected excitation light can still cause eye strain.
- **Electrical:** Keep liquids away from the iPad and the microscope base. If immersion oil or sample fluid contacts the electronics, disconnect power immediately and notify the lab manager.
- **Physical:** The microscope is heavy. Use both hands when rotating the platform between upright and inverted modes. Objective lenses and condensers are precision optics; handle with care.

5.1. Required PPE

- ☐ Safety glasses (especially during fluorescence mode)
- ☐ Lab coat
- ☐ Nitrile gloves (when handling biological specimens or immersion oil)

5.2. Emergency Procedures

If a biological spill occurs on or near the microscope, clean and disinfect per [SOP-L002 Laboratory Safety](#). If immersion oil contacts the iPad or electronics, power off immediately and contact the lab manager before attempting cleanup.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Echo Labs Revolve microscope	With iPad retina display
Objective lenses	4x, 10x, 40x, 100x (oil immersion)
Low-magnification condenser	For 4x through 60x objectives
High-magnification condenser	For 100x oil immersion objective
Fluorescence LED module	Excitation wavelength TBD nm
Phase contrast rings	PhL, Ph1, Ph2, Ph3 (matched to objectives)

6.2. Consumables

Item	Notes
Microscope slides	Standard 25 x 75 mm
Cover slips	No. 1.5 thickness (0.17 mm)
Immersion oil (Type A or NF)	For 100x objective only
Lens cleaning solution	Optical-grade; for objectives and condenser
Lens cleaning tissue (lint-free)	Do not use Kimwipes on coated optics
Compressed gas duster	For removing dust from the optical path
Flash drive or cloud account	For exporting captured images

7. Procedure

7.1. Microscopy Modes Overview

The Revolve supports four imaging modes. Select the mode appropriate for the specimen and analysis:

Mode	Best For	Staining Required?
Bright field	Stained specimens, gross morphology	Recommended
Phase contrast	Unstained transparent specimens, live cells	No
Fluorescence	Specimens with fluorescent labels or autofluorescent pigments (e.g., cyanobacteria)	Fluorophore-dependent

Mode	Best For	Staining Required?
Dark field	Fine structures, edges, unstained specimens	No

7.2. Starting the Microscope

1. Press the power button on the top left corner of the iPad (Echo Labs, 2016).
2. Swipe to unlock. The overhead microscope light should turn on and a live image should appear on the screen.
3. If the light does not turn on or no image appears, open the **Echo Labs** app from the home screen
4. If the screen reads “Light Off,” tap **BF** at the top of the screen to enable bright field illumination
5. Load the prepared slide into the stage clips (upright mode) or place the culture dish on the stage (inverted mode)

7.3. Rotating Between Upright and Inverted Mode

! Important

This is the most mechanically delicate operation on the Revolve. Follow each step carefully to avoid damaging the objectives or condenser.

1. Rotate the nosepiece to move all objective lenses out of the optical path
2. Unscrew the stage and carefully lift it away from the microscope body without contacting the condenser
3. Push the iPad flat so it is out of the rotation path
4. Press the release button on the upper back of the microscope body
5. Rotate the microscope body 180 degrees counterclockwise until it clicks into place
6. Reinstall the stage and tighten the mounting knob until secure
7. Rotate the desired objective lens back into position and realign the iPad

7.4. Using Bright Field Mode

1. Confirm that **BF** is selected on the screen
2. Place the slide or specimen on the stage
3. Start with the lowest magnification objective (4x) to locate the region of interest
4. Use the outer coarse focus knob (to the right of the iPad) to bring the specimen into approximate focus, then use the inner fine focus knob to sharpen the image
5. Use the stage translation knobs to move the slide: the lower knob moves the stage left/right, the upper knob moves it toward/away from you
6. Increase magnification by rotating the nosepiece to the next objective (10x, 40x)
7. Adjust brightness, contrast, and color balance using the sliding controls on the bottom right of the screen. Adjust the physical light intensity using the flat knob on the bottom right of the microscope body.

7.5. Using Phase Contrast Mode

Phase contrast converts differences in refractive index into differences in brightness, allowing transparent, unstained specimens to be visualized.

1. Set up the specimen in bright field mode first and bring it into focus
2. Select the phase contrast ring that matches the current objective:

Objective	Phase Ring
4x	PhL
10x	Ph1
40x	Ph2
100x	Ph3

3. Tap the **PH** mode selector on the screen (if available) or manually rotate the condenser turret to the matching phase ring
4. Fine-tune focus and centering as needed

Note

Phase contrast images may exhibit a bright “halo” around large objects (positive phase contrast) or a dark edge (negative phase contrast). This is a normal optical artifact caused by diffracted light passing through the phase ring. A “shade-off” effect, in which homogeneous regions appear at the same intensity as the background, is also expected.

7.6. Using Fluorescence Mode

1. Tap **FL** at the top of the screen to switch to fluorescence mode
2. The fluorescence LED will illuminate automatically
3. Place the specimen on the stage and focus using the same procedure as bright field
4. Minimize illumination time to reduce photobleaching. Locate the region of interest in bright field first, then switch to fluorescence only for image capture.

Warning

Fluorophores lose their ability to fluoresce over time when illuminated (photobleaching). Work quickly, minimize unnecessary excitation, and consider using photoprotective mounting media when possible.

7.7. Using Dark Field Mode

Section TBD. Consult the lab manager for dark field setup procedures until this section is completed.

7.8. Capturing and Exporting Images

1. To capture an image, tap the white camera shutter button on the left side of the screen
2. Enable the **anti-shake** feature (hand icon below the shutter button; it should turn orange when active) to reduce vibration blur
3. To review captured images, tap the gallery icon (square) at the top left of the screen. Tap **Done** to return to the live view.
4. Export images via a flash drive connected to the iPad, AirDrop, or the cloud sharing option in the Echo Labs app
5. Use a consistent file-naming convention: YYYY-MM-DD_SampleID_Objective_Mode.jpg

7.9. 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
4. Rotate the 100x objective into position. The lens front element 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. Never allow oil to dry on the lens.
7. Clean the slide or dispose of it as appropriate

7.10. Replacing the Condenser

The Revolve has two condensers: one for low magnification (4x through 60x) and one for 100x oil immersion. Condenser swaps require care to avoid contaminating or scratching the optics. Consult the lab manager before performing this procedure for the first time.

Detailed replacement steps TBD; to be documented with the lab manager.

7.11. Shutdown and Daily Maintenance

1. Remove the specimen from the stage
2. Rotate the nosepiece to the lowest-magnification objective (4x) or to an empty position
3. If oil immersion was used, clean the 100x objective immediately
4. Wipe any sample residue or oil from the stage with a Kimwipe dampened with lens cleaning solution
5. Close the Echo Labs app on the iPad
6. Press and hold the power button to turn off the iPad
7. Cover the microscope with the dust cover

8. Quality Control and Acceptance Criteria

8.1. Optics Cleaning

If specks or smudges appear in captured images that do not move when the specimen is translated, the optical path may need cleaning:

1. Do not attempt to clean internal optics without the lab manager present
2. For objective lens surfaces, apply a small amount of lens cleaning solution to lint-free tissue and gently wipe in a circular motion from center to edge
3. For dust on internal surfaces, remove the arm and use short bursts of compressed gas duster. Do not allow the compressed air canister to become cold, as it will deposit a film on the optics.
4. For persistent contamination, contact Echo Labs support or the lab manager

8.2. Image Quality Checklist

Before accepting an image for publication or analysis, verify:

- Focus is sharp across the region of interest
- Illumination is even (no hot spots or vignetting)
- Scale bar or magnification metadata is recorded
- Anti-shake was enabled during capture
- In fluorescence mode, confirm that photobleaching has not significantly degraded the signal

9. Waste Disposal

Waste Type	Disposal Method
Used immersion oil tissues	Standard trash
Used microscope slides (non-hazardous)	Broken-glass box if glass; standard trash if plastic
Biological specimen waste	Biohazard bag if applicable; autoclave before disposal
Spent lens cleaning solution	Standard trash (small volumes on tissues)

10. References

- Echo Labs. (2016). *Revolve microscope: User guide*. <https://www.echolabs.com>
- 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 the Revolve microscope independently, personnel must:

1. Read this SOP in full
2. Complete [SOP-L002 Laboratory Safety](#) training
3. Watch the Echo Labs training video at <https://www.youtube.com/watch?v=IcubmIsxa7w>
4. Observe a trained operator demonstrate upright/inverted rotation, all four imaging modes, and oil immersion setup and cleanup
5. Demonstrate proper startup, focusing, image capture, and shutdown under supervision
6. Be signed off by the PI or lab manager

12. Appendix A: Microscope Anatomy Quick Reference

Component	Function
iPad display (eyepiece replacement)	High-resolution live view and image capture
Arm	Connects optics to base; removable for optical path cleaning
Stage	Flat platform for specimens; translatable via X/Y knobs
Nosepiece (turret)	Holds and rotates objective lenses
Objective lenses	4x, 10x, 40x, 100x (oil); determine magnification
Condenser	Focuses illumination light onto the specimen
Coarse/fine focus knobs	Outer knob = coarse focus; inner knob = fine focus
Light intensity knob	Physical control on the base for illumination brightness
Phase contrast turret	PhL, Ph1, Ph2, Ph3 rings matched to objectives
Fluorescence LED	Excitation source for fluorescence imaging
Platform release button	On the upper back; allows 180-degree rotation between upright and inverted modes

13. Appendix B: Sections Requiring Completion

The following sections from the original SOP were incomplete or marked as placeholders. They should be completed with input from the lab manager:

Section	Status	Notes
Fluorescence LED wavelength	TBD	Record the excitation wavelength from the module specifications
Dark field mode procedure	TBD	Document setup and use
Condenser replacement steps	TBD	Document with the lab manager
Specific catalog numbers for consumables	TBD	Add slide, cover slip, oil, lens cleaner part numbers