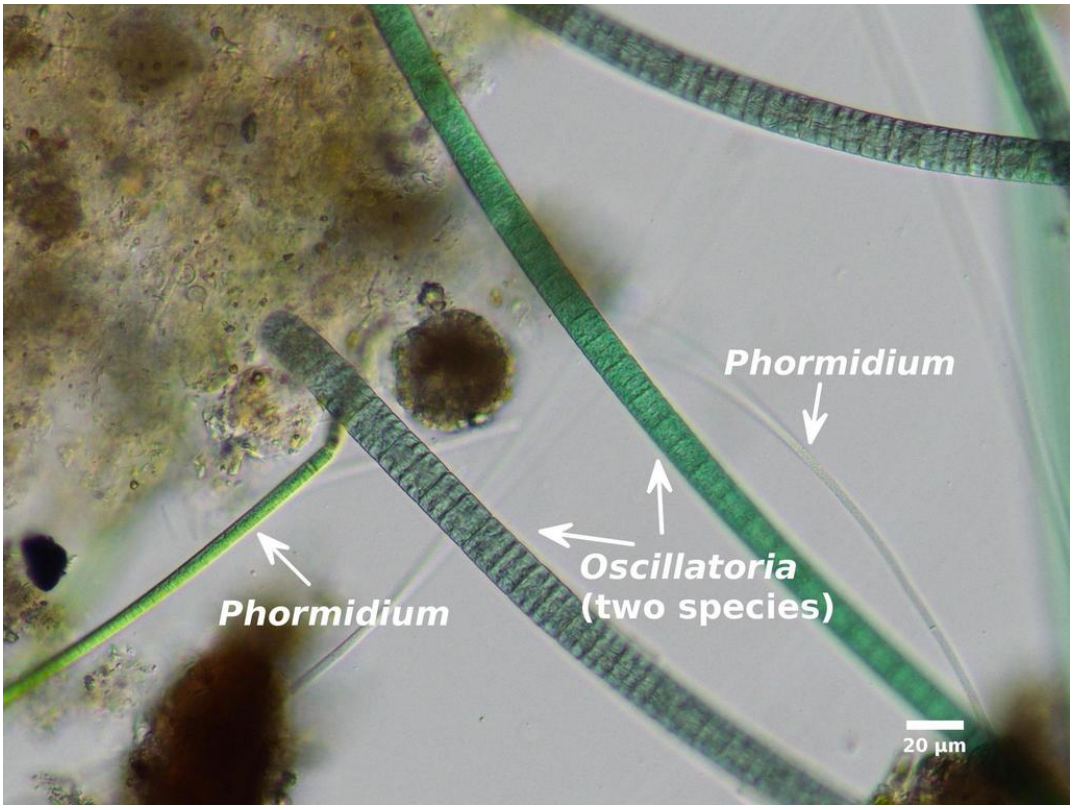
CyanoScope Quick Guide

Cyanobacteria Observation and Identification Handbook



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CyanoScope:
Cyanobacteria Observation and Identification Handbook

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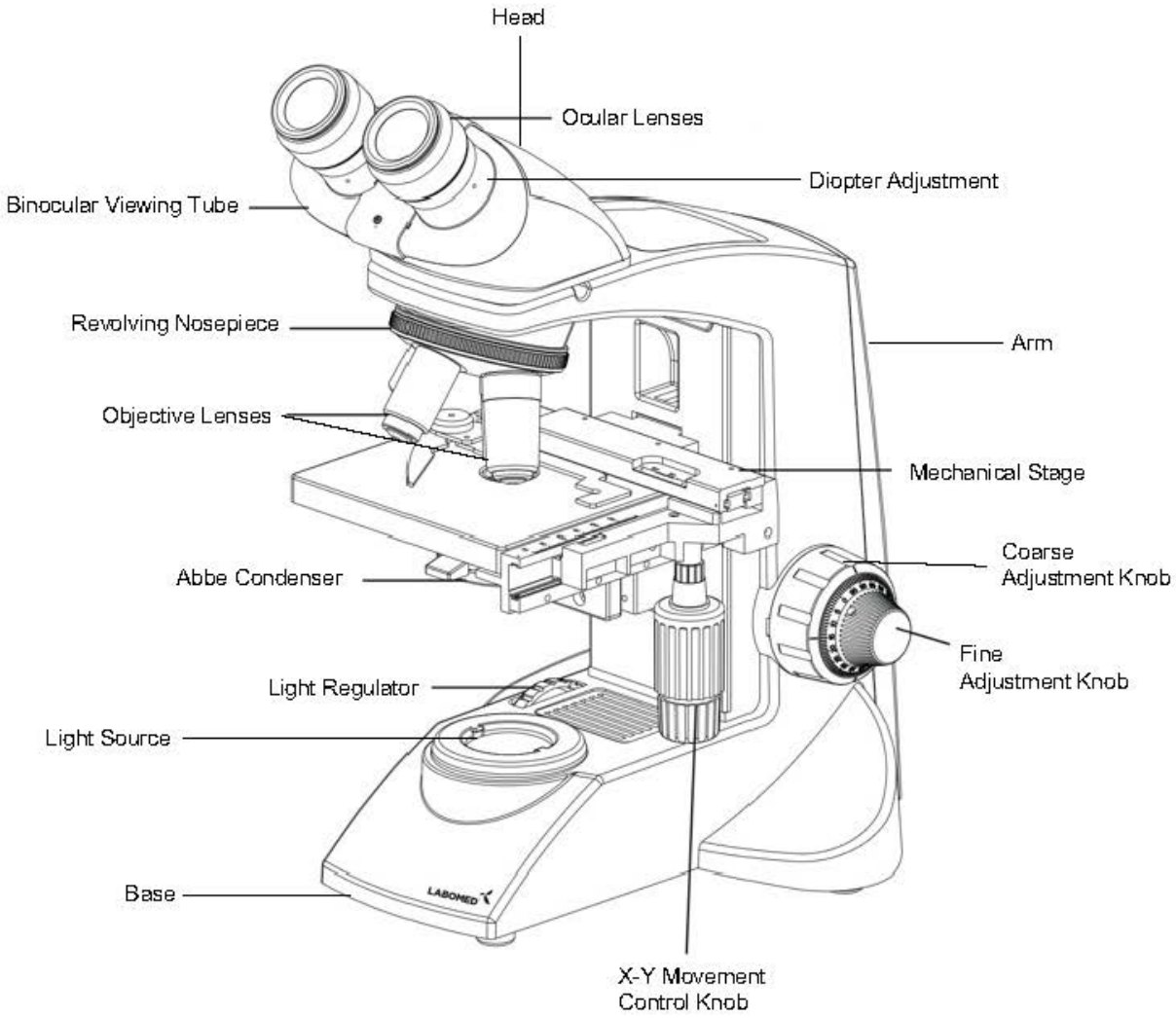
1. Recommended Materials

- **a.** Compound microscope (magnification ≥100x)
- **b.** Slides and coverslips
- **c.** Pipette
- **d.** Forceps
- **e.** Kimwipes
- **f.** Water sample (with suspected cyanobacteria)
- **g.** Reference slide (optional)
- **h.** Personal Protective Equipment (PPE) - e.g., waterproof gloves
- **i.** Digital camera or smartphone
- **j.** Your computer

2. Microscope Components



Microscope Component	Function
Head	*The upper part of the microscope that houses the ocular lenses and connects to the arm.*
Ocular Lenses	*The lenses closest to the eyes; magnify the image formed by the objective lens.*
Diopter Adjustment	*Adjusts the focus in an ocular lens to accommodate differences in vision between a user's right and left eyes.*
Binocular Viewing Tube	*Contains the ocular lenses and allows for comfortable viewing with both eyes.*
Revolving Nosepiece	*Rotating mechanism that holds multiple objective lenses, allowing for easy switching between magnifications.*
Objective Lenses	*Lenses closest to the specimen, magnify the image and determine the resolution and magnification level.*
Arm	*Connects the head to the base and provides support and stability.*
Mechanical Stage	*Platform where the specimen slide is placed, allowing precise movement in two dimensions.*
Abbe Condenser	*Concentrates and focuses the light onto the specimen for better resolution.*
X-Y Movement Control Knob	*Controls the movement of the mechanical stage in the X and Y axes for precise specimen positioning.*
Coarse Adjustment Knob	*Used for initial focusing by adjusting the distance between the specimen and objective lenses.*
Fine Adjustment Knob	*Allows for precise focusing by making small adjustments to the distance between the specimen and objective lenses.*
Light Regulator	*Controls the intensity of the light source to adjust brightness.*
Light Source	*Provides illumination for the specimen.*
Base	*The bottom part of the microscope that provides stability and support.*

3. Getting Started

a. **Note:** Do not touch the lenses or other optical components with bare fingers to prevent smudges and scratches. Fingerprint oils can damage the specialized coatings on lenses.

b. **Setup:** Place the microscope on a stable surface and ensure it has a charged battery or power source. Vibration of the base will make microscope use difficult.

c. **Microscope Use**

i. Adjust the eyepieces to match your interpupillary distance for comfortable viewing. View through the eyepieces should be comfortable and clear.

ii. Begin with the lowest magnification objective lens (usually 10x) and center the slide on the stage.

iii. Use the coarse adjustment knob to bring the specimen into rough focus, then use the fine adjustment knob for sharp focus. Become familiar with the direction that the stage moves when using these focusing controls. If the objective contacts the surface of the slide it can break the slide or damage the objective, or both.

iv. Increase magnification gradually by rotating the turret to the next higher-powered objective lenses; refocusing and recentering the specimen as necessary.

v. Adjust the light intensity using the abbe condenser and light regulator to optimize illumination and contrast for clear observation.

vi. Use the mechanical stage controls to navigate across the specimen slide for thorough examination.

d. **Care and Maintenance**
    i. After each use, clean the lenses, stage, and other surfaces using lens paper (e.g., a Kimwipe) and clean water or breath condensation to eliminate dust, oil, and debris. Do not use paper towels, facial tissues, or any chemicals.
    ii. Power off the microscope and unplug it from the power source.
    iii. Store the microscope in a clean, dust-free, low-humidity environment. Keep it covered with the dustless fabric cover provided.
    iv. Replace the microscope bulb if it appears dim or burns out.
    v. Follow the manufacturer's guidelines for any additional maintenance procedures or troubleshooting. The manual is available online: https://clemson.box.com/s/qfuhz1ene8pg0m3kl32yg4r1i6lwuir6.

4. Assess Sample Viability

a. **Age and Storage:** Cyanobacteria may begin to degrade after 24 hours of storage at room temperature, or sooner if stored in hot/sunny locations. If possible, store samples in a cool, dark environment (e.g., refrigerator) to maintain viability and examine the sample as soon as possible after collection.
b. **Visual Inspection:** Examine the sample for any signs of contamination, degradation, or unusual growth. Healthy cyanobacteria cultures typically appear green or blue-green in color, with uniform distribution and minimal debris.
c. **(Optional) Preservation:** If the sample cannot be examined quickly then it may be preserved for later use. Add a few drops of an iodine-based antiseptic to the sample container until the water has a 'tea' colored appearance. Recheck the sample after a few hours and if the color has faded, add more iodine solution until it maintains the 'tea' color. Keep the sample out of sunlight or fluorescent lights as the chemical is photoreactive. Alternative preservatives can be purchased or made specifically for this kind of laboratory application.

5. Slide Preparation

1) Clean the slide. Even brand-new slides often contain some residue or dust and should be cleaned with a mild detergent and/or a moist Kimwipe. Use caution to avoid breaking the slide.
2) Place the clean slide on your work surface.
3) Use a pipette to transfer a small amount of the sample, including suspected cyanobacteria cells, onto the slide.
4) Include just enough liquid to fill the space between the cover slip and the slide, but not so much that the coverslip slides around.
5) If needed, carefully spread out the cells to prevent clumping using forceps or dissecting needles.
6) Gently lower the coverslip onto the sample to avoid crushing the cells.
7) Take care to limit air bubbles under the cover slip. A few bubbles are common; however, if there are excessive bubbles, clean the slide and start over.

6. Camera Use

a. Setup (for ACCU-CAM 500 EP)

i. Charge the camera or connect it to power using the provided cord.
ii. Remove one optical lens and replace it with the camera.
iii. Connect the camera cord to your laptop.
iv. Click on the ACCUVIEW LITE software icon and, if necessary, download it onto your computer.
v. Once installed, open the ACCUVIEW LITE software and ensure it automatically connects to the microscope.
vi. Set up a dedicated folder on your computer for cyanoscope images.

b. Capture Image

i. Scan the slide to look for potential cyanobacteria cells then take a photograph of areas of interest.
ii. If using the ACCU-CAM 500 EP: Capture images by clicking "snap" when the desired portion of the sample is in view.
iii. If dedicated camera is unavailable, a smartphone can be used following the Morrison Technique:
    1. With your left hand, grip the left ocular of the microscope steadily.
    2. Use the last three fingers of your left hand to brace against the left ocular for stabilization.
    3. Hold the smartphone between the remaining fingers of your left hand and your right hand.
    4. Position the smartphone camera lens directly over the eyepiece of the microscope.
    5. Adjust the smartphone camera settings, such as focus and exposure, for optimal image quality.
    6. Carefully align the smartphone camera with the eyepiece to capture the image of the cyanobacteria cells.
    7. Steadily hold the smartphone to prevent blurring and ensure a clear image (Figure 1).
    https://www.youtube.com/watch?v=cfd9ViHBlR4

c. Shutdown

i. Close the ACCUVIEW LITE software.
ii. Disconnect the camera cord from your laptop.
iii. Remove the camera from the microscope and replace it with the original optical lens.

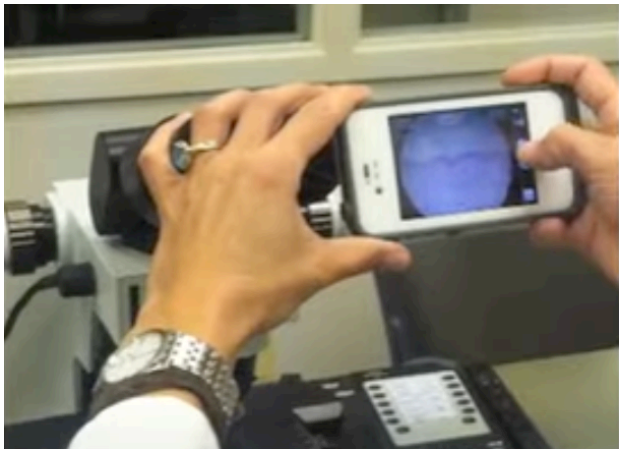


Figure 1. Example of the Morrison Technique hand placement to stabilize a cell phone and capture a photograph through a microscope. Image Credit: YouTube Annie Morrison.

7. Clean Up

a. **Sample Disposal:** Disposal of samples depends on their condition. Samples that do not contain cyanobacteria may be discarded directly down a drain to the sanitary sewer or as compost. If the sample contains cyanobacteria but no other chemicals, add 1 tsp bleach to the sample (without Lugol's) and let sit overnight, then dispose down a sink with lots of water. If the sample has been preserved with a chemical preservative do not add bleach before disposing of down a sink with lots of water.

b. **Slide Cleaning:** Carefully wash slide and cover slip with mild detergent and dry with Kimwipe before storing for reuse.

8. Distance Diagnostics

a. **Note:** *This is a pilot program that is being provided on a trial basis for no additional cost (beyond charge for sample id). It is being offered in limited counties and is dependent on Agent availability.*

b. **Sample Intake:** Extension staff should intake algae/cyanobacteria sample as normal – e.g., client should complete form, pay sample id fee, and – after making a slide – the remaining sample should be shipped to the Clemson Plant & Pest Diagnostic Clinic. See HGIC 1889 Submitting an algae sample for identification (https://bit.ly/AlgaeSample) for more information.

c. **Sample Evaluation:**
If sample is suspected to be cyanobacteria and Agent has availability, follow these steps:
   i. Encourage the client to email you pictures of the pond and algae/cyanobacteria in the pond or waterbody.
   ii. Follow instructions in Section 1-6 to produce pictures of suspected cyanobacteria cells.

d. **Picture Sharing** (Box)
   i. Upload all available pictures (of pond, plant, and/or cells) to HAB_Sample_Identification shared Box folder. (https://clemson.box.com/s/639v92l3pisztjbwazzv1ewvm0bq0wf7).
      1. Requested file system: Individual folder for each client, using client's name in this format: LastName_FirstName
      2. If repeat client (i.e., a Box folder already exists for them), create a subfolder by date using this naming convention: YEAR.MO.DA (ex: 2024.05.21).
      3. Add available pictures of the pond, plant/algae, and suspected cyanobacteria cells (from microscope).
      4. If client has approved of Clemson University's use of the photos for educational purposes; please include a pdf of relevant email in the Box folder. Please include preferred name that should be used for image credit (or "anonymous", "SC resident", etc.).

e. **Information Sharing** (Salesforce)
   i. Log into Salesforce.
   ii. Create a client "Contact" record (if not already in the system).
   iii. Create an "Interaction" that describes the situation. Helpful information could include: pond details (size, depth, location), description of problem (when did it start, how much of pond is covered, etc.), and use of the pond (e.g., recreational, livestock, stormwater).
      1. Include a link to the Box folder with the associated pictures.
      2. Tag relevant individuals as "Referred Specialist(s)" or "Referred Agent(s)" Required: John Hains. Suggested: Deb Sahoo, Sarah White, and Heather Nix. This will auto-generate an email to tagged individuals and they can log-in to review details of the interactions.
      3. Salesforce can be used for internal communication between Agents-Specialists-etc., and lab staff can provide a link to their final reports to ensure Agents have access to that information.