

## CyanoScope Plankton Sampling and Counting Protocol

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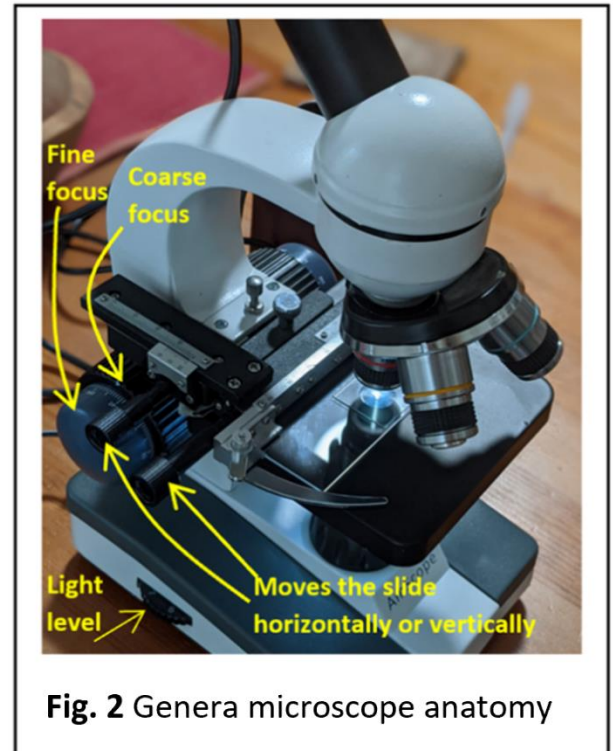
- 1) **At the lake, collect your plankton net sample:**
  - a. Lower the plankton net to a depth of 2 meters (each piece of tape marks 1 meter, stop lowering when the 2<sup>nd</sup> tape is at the surface of the water). Steadily draw the plankton net back up and out of the water. Rinse any material that may be stuck to the side of the net by dipping the plankton net back into the water, making sure **NOT TO** lower it past the metal ring at the top, and swiftly draw it back out of the water. Repeat this 2-3 times, or until the sides of the net look clean.
  - b. Next, tip the container (at the base of the net) to allow some of the water to freely flow through the net - this will help concentrate your sample as water flows out freely, but the plankton will not. Pour the sample into your sample bottle by orienting the hose over the bottle and releasing the clasp.
  - c. **Finally, repeat Steps A+B** so that you have collected a total of **TWO** plankton tows.
- 2) **Take your sample back to the LSPA.**
- 3) **Concentrate the sample in the sink:** Securely re-pinch the clasp on the plankton net hose to make sure it's securely tightened. Working over the sink, pour the contents of the sample bottle back through the plankton net to concentrate it again. Tip the container to allow even more water to flow through the net, concentrating the sample even more. Aim to concentrate it such that the water level in the container is just above the metal weights.
- 4) **Pour sample back into the sample bottle:** Align the hose back over the sample bottle and release the clasp to pour your highly concentrated sample back into the sample bottle.
- 5) **Pour sample into the Graduated Cylinder:** Prepare a graduated cylinder on a flat surface and place a funnel in the opening. Carefully pour the sample from the sample into the graduated cylinder, making sure NOT to overflow. If needed, pour the excess sample into a second graduated cylinder.
- 6) **Record the volume:** Determine the volume of your sample in the graduated cylinder – hold the graduated cylinder eye-level and record which line the liquid reaches (you may use 0.5 increments if the water is between two lines). If you used two graduated cylinder, make sure to add the two volumes together. Record this volume in the CyanoCounter webpage.
- 7) **Pipette the sample into the grided microscope chamber:** Using the plastic pipette provided, gently dip it up and down in the graduated cylinder to make sure the sample is evenly mixed. Then use the pipette to draw up sample until the water is ~halfway filling the pipette (hold the pipette above the sample, fully squeeze the top with your fingers, then dip the pipette at least halfway into the sample, start releasing your squeeze gradually to draw sample into the pipette until it is filled about halfway, then pull the pipette back out of the graduated cylinder making sure to holding the squeeze). Carefully squirt the sample from the pipette into the grided microscope slide chamber by squeezing harder to release its contents). The chamber holds exactly 1 ml, so keep filling until the liquid reaches the edge of the chamber. If the water doesn't fill the chamber, fill the pipette again and repeat until

the chamber is just filled to the edge. Any leftover sample in the pipette can be squirt back in the graduated cylinder.

- 8) **Add cover slip:** Place a cover slip onto the top of the chamber and use a paper towel to clean any spilled liquid from the side.
- 9) **Place under microscope:** Place the grided slide under the microscope and adjust the focus. To do this, scroll around the slide adjusting the focus back and forth until you recognize a type of cyanobacteria, and get that into focus. That's how you know you're in the correct plane of focus.
- 10) **Count cells in 3 randomly selected rows:** Start at the top left of the slide, skip the first row, then count any 3 random rows. For each row you count, start at the left or right edge and count all of the cells (or colonies) as you see them. When you reach the end of the row, move down the slide to a new row.
- 11) **Use the [CyanoCounter](#) to keep track of each type of cyanobacteria:**
  - a. The CyanoCounter app allows you to keep track of the number of each type of cyanobacteria you encounter on your slide – after you're finished counting, you can simply click "Export Data" to download all of your count data to the computer, where it is automatically saved
  - b. To access the CyanoCounter, open the internet on the LSPA laptop and it will automatically appear as your homepage
    - i. URL: <https://jvthaney.github.io/cyanocounter/>
  - c. **Follow the instructions on the CyanoCounter webpage** to log your plankton tow information and start your counting
- 12) **FOR DETAILED INSTRUCTIONS ON MICROSCOPE SETUP/USE**, see the next page.

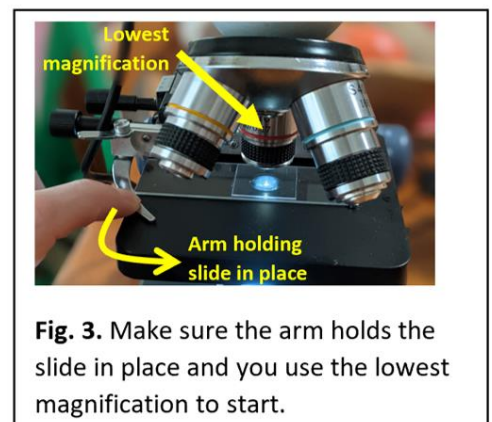
## 1. IDENTIFYING ON THE MICROSCOPE

1. **Make sure the AmScope software is open** on the computer (if it is not already open, double click the AmScope icon on the desktop)
2. **Make sure the microscope is ON** (switch on the back) and the light is ON (lever on the side of the base, Fig. 2)
3. **Place the slide with cover slide on the stage of the microscope** (Fig. 3) and press down on the arm of the slide-holder to make sure it is securely pressing against the slide (if the arm is not pressed down, it can snap closed over the top of the slide)
4. **On the upper left-hand side of the screen under “Camera List”, click the only camera listed** (it will likely start with “MD...”). This will tell the program to connect to the microscope camera. A box should appear showing a live stream of the microscope slide
  - a. Note: it may appear washed out for a few moments as the autofocus and light levels on the camera adjust



**Fig. 2** Genera microscope anatomy

5. **Use the lowest magnification (red line indicated in Fig 3).** The plankton you are counting should be identifiable at this magnification.
6. **Now adjust the focus** on the slide using the fine and coarse focus knobs (Fig. 2).
  - a. A helpful trick to find the right “plane of focus”: use the grid lines on the slide as a point of reference for the focus – they should be in focus if you are at the right level.



**Fig. 3.** Make sure the arm holds the slide in place and you use the lowest magnification to start.

7. **Now it's time to look for cyanobacteria:**
  - a. Navigate to the upper left corner of the slide
  - b. Skip the first row
  - c. Now pick any 3 rows below this to count
  - d. For each row, start at the edge and move horizontally across the row. When you reach the other side, choose another random row below that and repeat by moving horizontally in the other direction.
8. **Snap pictures using the AmScope software:**

- a. Please take a picture the first time you encounter each type of cyanobacteria during every counting session – that way, I have a sense for what you are identifying as each type that day
    - i. Also please take pictures if you're unsure whether something is cyanobacteria – this allows me to identify any unknowns from the images later – and feel free to take pictures of anything else you see of interest!
  - b. Take a picture this by clicking the “Snap” button on the left-hand side of the AmScope program (in the “Capture & Resolution” section)**
    - i. This will take a picture of the current view and place that image in a tab above your live stream.
  - c. After you have “snapped” the picture, make sure you click the “Live Stream” tab again to get back to the live stream view of the slide**
- 9. Save the images after you're done counting:**
- a. **When you're done counting your 3 rows, save the images in AmScope:**
  - b. Select File → Batch save. Under “File prefix”, type the date and your name/initials (e.g., 21June21\_JessTH). Click OK. This will save all your images under your name and date. You can now close the AmScope program.