



Phytoplankton Microscopy Lesson

Grade Level: 6th-12th grade

Subject: Biology, Marine Science

Recommended length of time for unit: 90 minutes each

Overview: This activity will be taking part on the second day of Clam Camp, after the students have collected their individual phytoplankton samples at the designated sample location. Each student will have their own water sample, microscope kit, genera identification booklet, and data recording sheet, and field notebook.

Objectives/Goals:

- Students will be able to identify in the three common harmful algal bloom phytoplankton genera: Alexandrium, Pseudo-nitzschia, and Dinophysis. Students should also have the ability to match phytoplankton with the corresponding biotoxin and resulting illnesses.
 - Students will be able to name the two classifications of phytoplankton that are being studying during Clam Camp: dinoflagellates and diatoms. They will also provide differences between the types and tips for classifying each.
 - Students will explain how toxins are transferred from phytoplankton to shellfish to humans and other animals.
 - Students will use a microscope and the common southeast Alaska identification booklet to identify and record plankton that they find respective samples.
 - Students will be able to set up their own microscope slide.
 - Students will show an understanding of harmful algal bloom myths such as blooms are always visible (red tide), if there was a bloom you would see sick animals in the area, and that blooms only occur in the summer.
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Part One- Phytoplankton introduction

Materials: Foldscope kit for each student (foldscope, instructions, LED light, glass slide, sticker cover), field notebook and pencil, southeast Alaska plankton ID booklet.

COVID safety: The students will have their own paper Foldscope microscope kits and will socially distance, in the field, while wearing masks.

Phytoplankton Identification: Students will be given Southeast Alaska Tribal Ocean Research's (SEATOR) common phytoplankton of southeast Alaska identification booklet. The booklet includes brief descriptions and image examples for common phytoplankton genera. There is an observation and data tracking sheet in the back of the booklet which they will use to tallying genera in the subsample. A Sitka Tribe staff member will demonstrate how to use the microscopes and will explain booklet and data sheet to the students before they conduct their sample microscopy. Sitka Tribe staff will also cover the differences between diatoms and dinoflagellates, genera characteristics, and helpful hints for identifying genera.

Part Two- Phytoplankton introduction

Purchasing materials: Students will each receive a Foldscoptes paper microscope kit in individual pouches. LED lights were also purchased separately from the Foldscoptes; these lights were an add-on items and are not part of the standard kits. Glass slides can be ordered from Foldscoptes, but any standard glass microscope slide can be used. Each student should have at least one glass slide. Sticker water sample slide covers were ordered from Foldscoptes. Standard glass or plastic slide covers should not be used because the slides do not lay flat in the microscope. Each student should be given approximately three water slide covers from the master sheets, depending on the resources available. And each student should have cotton swabs and/or lens paper for cleaning off their slide and microscope lens. Students will also need at least one disposable plastic pipette. Foldscope paper microscopes selected so microscopy can be done in the field and to adhere to COVID safety measures.

Assembling Foldscope paper microscopes: <https://www.youtube.com/watch?v=0iRCceGCGus>

If there is internet at the field location, and a capacity for video showcasing (including possibility of the students watching on their own internet capable devices), students should watch the Foldscope YouTube tutorial video above for visual set up instructions.

An abridged assembly/user guide should be included with each student kit. The Foldscope user guide is also provided online and it includes instructions for setting up slides, moving slides, viewing slides and photographing slides with a phone's camera:

<https://www.foldscope.com/user-guide>

Foldscope microscopes can be purchased pre-assembled at an additional cost. Sitka Tribe staff may assemble microscopes if time and capacity allow, and with consideration for COVID safety (gloves, disinfecting parts when possible).

The paper microscope pieces are die-cut. The pouch also includes four stacked magnetic couplers in a small plastic bag. Detaching the colored, paper, die-cut microscope pieces must be done cautiously as the pieces can easily rip and especially around tabs and center-cut holes.

Remove the white centers from the four main pieces. Besides the white, rectangular paper slide pieces, there are four main parts of the microscope. The lens stage piece is a cross or lowercase t-shape. The panning guide piece is a squat rectangle with two uppercase T-shapes on the top and bottom with slits on the other sides. The focus ramp piece has tiny column cutouts leading to two rectangular shapes with centers cut out. Students should be careful when detaching the focus ramp because of the small, fragile column shapes on one end. Finally, the slide stage is a longer rectangle with a center rectangle cutout and two small tabs on the top and bottom.

Once the students have pulled all the die-cut pieces out, they should carefully open the small plastic bag containing the four stacked magnetic coupler pieces. The pouches also include a larger plastic bag with stickers. Remove three of the rectangular magnetic pieces to reveal one circular piece. The small circular piece is the lens. Students should now locate the center circle cutout of their lens stage piece (the cross shaped piece). One rectangular magnetic couple piece attaches to the front and the other attaches to the back. The third rectangular magnetic coupler piece will be used to attach a phone camera, so it can be safely set aside for later use.

Starting with one of the rectangular magnetic couplers, locate the gray stripes around the perimeter of the circle cutout. The stripes are also magnetic so the gray side of the magnetic coupler will attach to the cutout circle of the lens stage and the respective circles from each piece should align. Tuck the corners of the coupler into the slots on the die-cut piece. After the front coupler has been attached, flip the lens stage over and attach the back magnetic coupler using the same method except this time the cutout circle is located on a flap (not in the center of the cross). It has black lines around the perimeter. This time attach the black magnetic coupler, which also has a smaller circle. The coupler corners need to be tucked into the die-cut piece, just like they were with the front coupler.

Fold the flap, with the now attached back magnetic coupler, along the dashed line and tuck the three small flaps. Now locate the sticker bag and take out the translucent, double-sided ring stickers sheet. The ring stickers will be used to attach the circle lens piece. Peel a sticker off the sheet but keep the white cover on one side of the sticker to help with transfer. Flip the lens stage back over and attach the ring sticker to the black side of the front coupler (in the center of the cross), making sure to align the sticker with the black circle shape of the coupler. Gently press on the sticker before peeling off the white sticker cover. Attach the lens to the ring sticker with the silver side down. The lens will also magnetically attach to the coupler, which will help ensure the correct lens side was attached.

Next, pick up the focus ramp die-cut piece, which is the shape with small columns and two rectangles. This piece has eight dashed lines. With the gray side facing up, fold each dashed line towards yourself. After all creases have been made, roll the sections along the crease, starting with the shortest end. Smooth down the sides to make sure the edges are lined up. One end of this folded rectangle should be thicker and the other thinner. Weave the focus ramp into the slits of the lens stage. These slits are on the sides of the center circle and attached front coupler and lens. Go through one slit, weave behind the lens and front coupler (the silver side of the coupler, without the lens) and then go back through the other slit, so the two focus ramp ends are on the same side.

Once the focus ramp has been inserted into the lens stage, pick up the slide stage. The slide stage is the longer, blue rectangle shaped piece with two yellow small tabs. With the blue side up, fold the yellow tabs over and tuck them into the slits beside them so that the yellow is no longer visible. One side should now be all blue and the other side should be all yellow. You will notice that there are small arrows on the yellow side, beneath the folded tabs, this is where the slide will be attached.

Find the final piece, the squat rectangular panning guide, and make sure it is also on the yellow side. Weave the ends of the slide stage through the two slits on each end of the panning guide. The yellow side of the slide stage should still be facing up when attached to each end of the panning guide. Line the center cutouts of each piece up so there is an empty center.

It's time to assemble all the pieces! Take the paired panning guide and slide stage and turn them over to the blue side. Make sure the lens stage is also on its blue side. Place the lens stage on top of the panning guide. You should see the word "Foldscope" through the top window of the lens stage. Flip all the pieces over to the yellow side. You will now see eight red rectangles. Gently pull the red rectangles through the blue side, so that the tabs are holding all the pieces together. You should still be able to move the lens up and down and from side to side.

Finally, take the flap with the other coupler (yellow flap on the blue side) and fold it with the yellow side of pieces up, along the four dashed lines in the following order. The first fold is on the line closest to the center coupler. Fold the flap towards yourself, over the coupler. The second fold is close to the first, along the small blue section of the flap (still on yellow side of pieces). Fold it down and towards you. The third line is further down the tab and should be folded down and towards you as well. The last fold is close to the flap's coupler, it should be folded up or away from you. Bring the folded flap coupler section to the center coupler. The pieces should magnetically attach. The Foldscope paper microscope is now assembled!

Setting up slide: Take a lens wipe or cotton swab and clean the lens and coupler pieces before and between each use, on both sides. Students should avoid touching these clean pieces while setting up their slide. Students must also clean their glass slide, with a lens wipe, and should avoid touching the slide or placing it on dirty, dusty surfaces after cleaning.

Each student will have their own preserved phytoplankton tow sample in labeled sample bottles, which they collected during the previous Clam Camp day. Next, they need to find the data recording sheet at the back of their SEATOR common southeast Alaska phytoplankton booklet guide. The students should transcribe the field sampling data from their sample bottle onto their data sheet or in their notes. Their phytoplankton observations will be shared and discussed with the group at the end of this activity.

Gently invert the capped bottle to mix the sample because the phytoplankton and algal materials can settle at the bottom, but the subsample needs to be from a homogenous mix. Find the water sample cover stickers. The cover stickers have two pieces. One half has a black ring, which is the bottom piece, the other half is a clear circle cover. Attach the sticker with the black ring onto the center of the glass slide. Using the disposable plastic pipette, extract a subsample and place approximately two drops onto the center of the sticker, within the black ring. Now, take the clear circle sticker cover and place it over the black ring and the water drops. Gently adhere the cover to the sticky black ring. The cover should slightly bubble over the drops. Cautiously flip the slide to test for leaks.

Pull back the folded-over microscope flap. Make sure the microscope is its yellow side. The smooth, non-sticker side of the slide should face down. Weave the ends of the slide through center folded tabs (arrows are beside tabs). The pieces of the microscope should still move up, down, and from side to side with the glass slide still securely attached. Fold the flap back over, covering the now attached slide. Flip the microscope over to the blue side to view the sample through the center eyepiece. Shine the LED light through the center eyepiece from the yellow side. A phone camera lens can be placed on top of the center of the eyepiece for photographing organisms.

Recording phytoplankton genera: Pan through the sample and identify any phytoplankton genera by using the booklet with genera descriptions and image examples. Record observed genera on the data sheet or in notes.

Part Three- Group discussion

Discussion: Students will be asked to share which phytoplankton genera they observed in their subsample, especially any harmful algal bloom genera. Staff will record and compile the student observations so the group can make conclusions about which phytoplankton genera were seen in the greatest abundance or most commonly among the group. The group should also discuss whether harmful algal bloom genera were observed, their abundance, the implications of their presence and quantity, and actions SEATOR takes when they are found in a sample.