

Magnified eyes on the HABs problem: inexpensive scopes project shows promise as rapid screening tool for water samples for elevated microcystin toxins

Authors: Valerie Aubley (HAB Harrier and Cornell student), Ilana Hill (Cornell student), Lydia LaGorga (Cornell student), Ruth Richardson (Cornell professor)

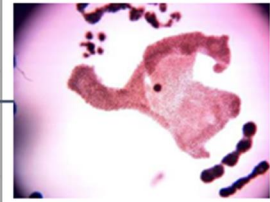
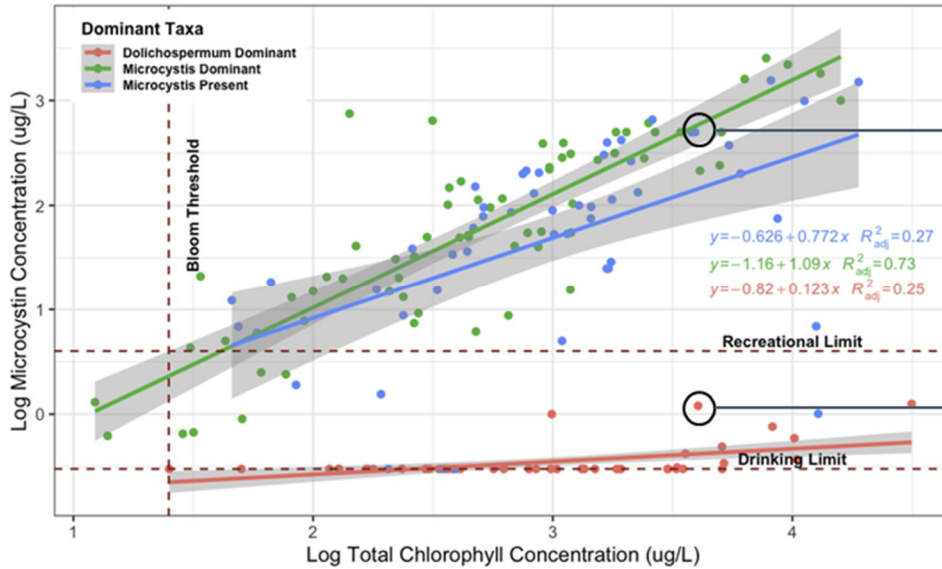
Introduction

Harmful algal blooms (HABs) have been on the rise in New York State, and Cayuga Lake experienced more blooms than any other NY waterbody in 2020. In fact, 10% of the HABs reported to the NYS DEC statewide in 2020 (94 out of 930) were from Cayuga Lake. Algal blooms are triggered by the presence of excessive nutrients like phosphorus, which spur the growth of the naturally occurring microbe cyanobacteria that congregate into colonies, creating the appearance of "pea soup" at the surface of the water. However, not all algal blooms are classified as HABs: only some types of cyanobacteria produce harmful toxins. This article reports on first steps to developing an inexpensive method for trained volunteers to rapidly determine HABs species and toxicity.

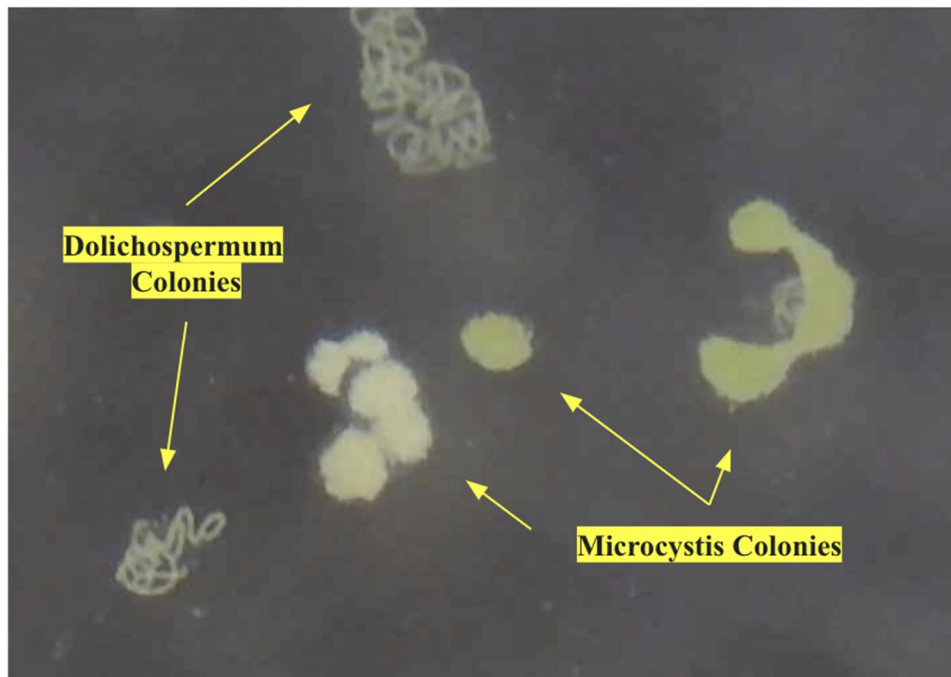
In order to address the growing issue of HABs on Cayuga Lake, the Cayuga Lake Watershed Network (CLWN), Community Science Institute (CSI), the Finger Lakes Institute (FLI), and Discover Cayuga Lake worked together to establish the HABs Harriers program, a seminal citizen science program in which community members serve as "HAB Harriers" who monitor sections of Cayuga Lake on a weekly basis, checking for and sampling blooms when they occur. The HABs Harrier program has successfully provided three years of data from the resulting HABs samples (2018-2020), from which some notable trends have emerged.

Key datasets include values measured by the Community Science Institute: chlorophyll A levels (measure of overall bloom level), microcystin toxin levels (MC), and microscopic confirmation of the presence of cyanobacteria. Cyanobacteria are the key toxin producing organisms in freshwater HABs (sometimes called cHABs). Microscopic identification can also be made as to which genera of cyanobacteria are present. By analyzing all cHABs from 2018-2020, it is clear that the high toxin blooms (>4 micrograms per L of microcystin) all have dense colonies of one particular genus: *Microcystis*.

Some blooms are seen on Cayuga Lake that are dominated by other genera, most notably *Dolichospermum*. These two types of colonial cyanobacteria have very different features under the microscope. Furthermore, samples of these cyanobacteria can be subjected to biochemical analyses for MC level measurement. However, the biochemical analyses for quantifying MC values requires an accredited laboratory and the use of an analysis called ELISA. These tests are both expensive (\$30-\$200) and can take up to a day or more to get results.



Microcystin Toxin Concentrations versus Bloom density for Blooms on Cayuga Lake. Composite 2018-2020 data from Cayuga Lake of log MC level versus Log ChlorA level for blooms overall and sorted by color according to which genera were dominant in microscopic views. Dolichospermum and Microcystis are the dominant genera present, with some samples having both present simultaneously. Regressions are color coded by series being fit. Right side: representative micrographs of colonies of Microcystis (top) and Dolichospermum (bottom) from dense bloom samples (chlorophyll A levels >1000). Data from CSI website: <http://www.communityscience.org/volunteer/harmful-algal-bloom-monitoring/cayuga-lake-habs-reporting-page/>



Dolichospermum bloom colonies vs Microcystis bloom colonies in a single sample as viewed with an inexpensive microscope kit. Dolichospermum colonies, which usually form thin, curly-q shaped with

nitrogen fixation cells called heterocysts, do not produce microcystin. Microcystis, which form globular, thick colonies, are the main culprits of microcystin production on Cayuga Lake. Sample from zone 3416 in southern Cayuga Lake, Bloom 1, September 2020.

Current Happenings

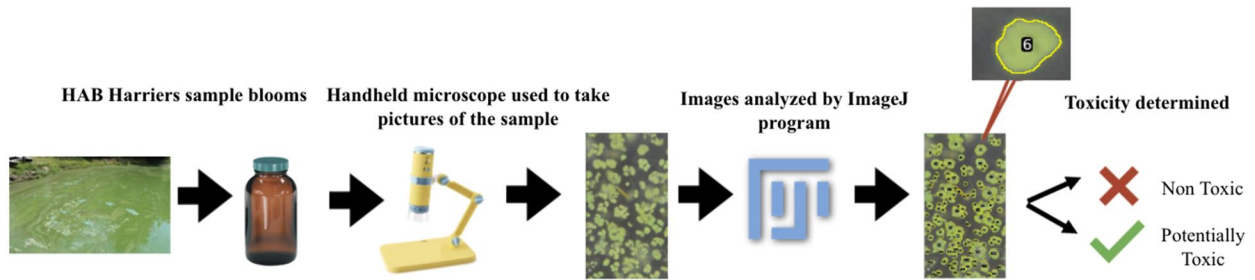
Inspired by the first two years of HAB Harriers data, a Cornell team of students under the direction of Associate Professor Ruth Richardson decided to do a trial as to whether inexpensive microscopes (\$40) could be used by trained volunteers to visualize how dense the colonies are in a water sample and to discern which genera are present by capitalizing on the distinct shape difference between the genera. This led to the creation of a HABs identification program using handheld microscopes, qPCR and automated image analysis to create an efficient, cheaper alternative to the current HABs identification method. Over the summer of 2020, we at the Richardson lab teamed up with volunteers from the HABs Harrier program to prototype this field kit identification method. Throughout the summer, Harriers who prototyped the Richardson Lab's field kit used the handheld microscope kit to take pictures of any and all blooms they observed in their monitoring section.

We were able to compile hundreds of images, capturing microscopy pictures for more than fifty HABs. We also used qPCR, a method of gene detection similar to those used for Covid-19 exposure testing, to analyze the toxin gene levels in the bloom microbial community.

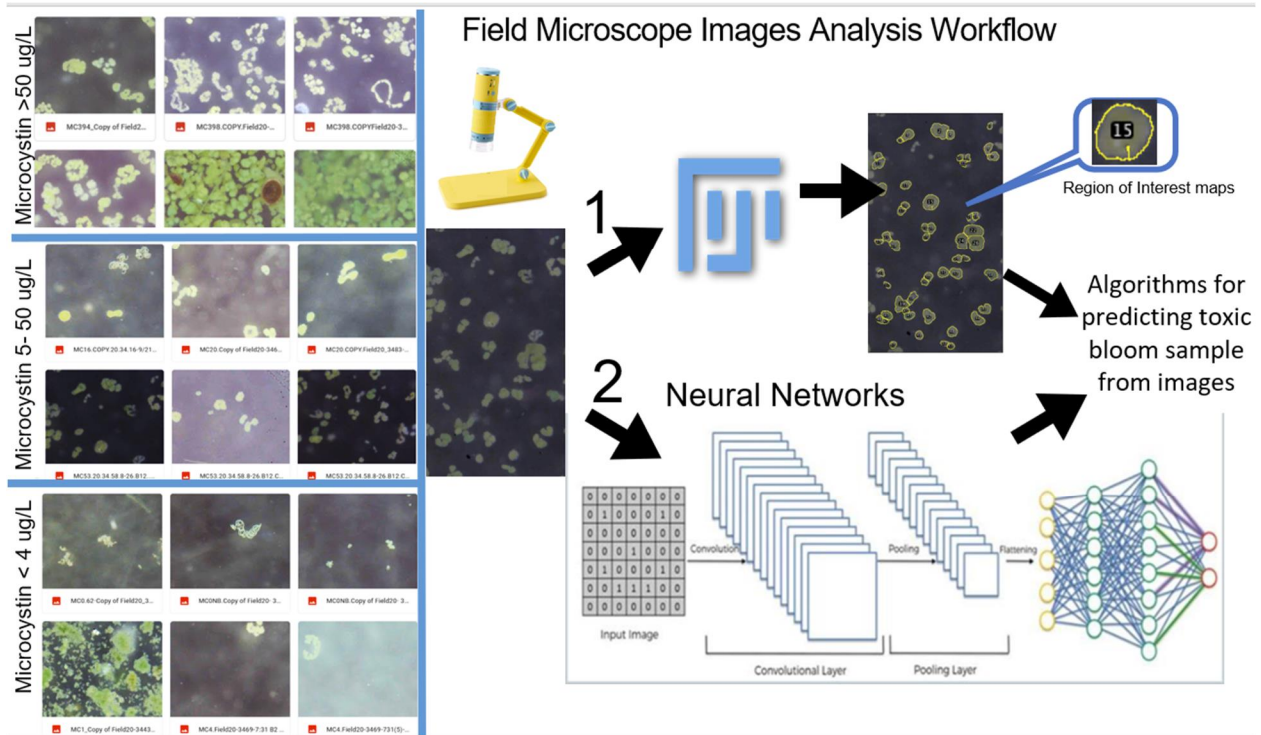
Our team is in the process of creating an image analysis system using the cheap scope images and the results of qPCR as rapid, inexpensive screens for the presence of high toxin cHABs. Using the software ImageJ, we parse through the colors, shapes and bloom density within a sample image to isolate *Microcystis* colonies. By running the sample images through the analysis, we aim to create a program that can discern toxin producing colonies from non-toxic ones, and present an expected MC value for each provided sample. The MC level attained from the image analysis is compared to the toxicity level results obtained from the qPCR tests, as a way to determine the effectiveness of the program.



Our team at Taughannock State Park collects water samples in the field to be analyzed using our field kit identification method and qPCR.



Flow chart Richardson Lab sampling and analysis overview.



Left: representative raw bloom images taken with the inexpensive microscopes grouped by level of microcystin toxin by ELISA assay. Right, example workflow for automated image analysis to predict level of toxicity of a water sample. Priority pathway is “1” with “2” as alternate/contingency. Either pathway takes less than one minute to analyze.

Future Plans

We aim to eventually expand our image analysis program to other Finger Lakes and upstate New York watersheds. We are currently working with groups on Canandaigua, as well as Cayuga Lake. The tool could conceivably be used in any water body with HABs issues caused by *Microcystis* colonies. As the image analysis program progresses, we will continue sampling blooms and relying on our HAB Harrier volunteers to help our research continue. We will also be continuing our field kit prototyping in the Summer of 2021 so be on the lookout for more information about volunteering -- we'd love to have more community members involved.

For more information see:

1. CSI HABs reporting page: <http://www.communityscience.org/volunteer/harmful-algal-bloom-monitoring/cayuga-lake-habs-reporting-page/>
2. [Map with micrographs/videos from the 2020 blooms](#)
3. Richardson lab website:
4. Beautiful images of cyanobacterial types (websites):
 - a. Algae, Cyanobacteria, and other aquatic objects: <http://cfb.unh.edu/phycokey/phycokey.htm>
 - b. Algae image library: http://snoringcat.net/AlgaePage/index.php?path=Algae//Bluegreen_Algae
 - c. Microscope UK (includes more than algae, eg rotifers, zooplankton): <http://www.microscopy-uk.org.uk/>

Interest Form if you want to sign up for our 2021 monitoring project:

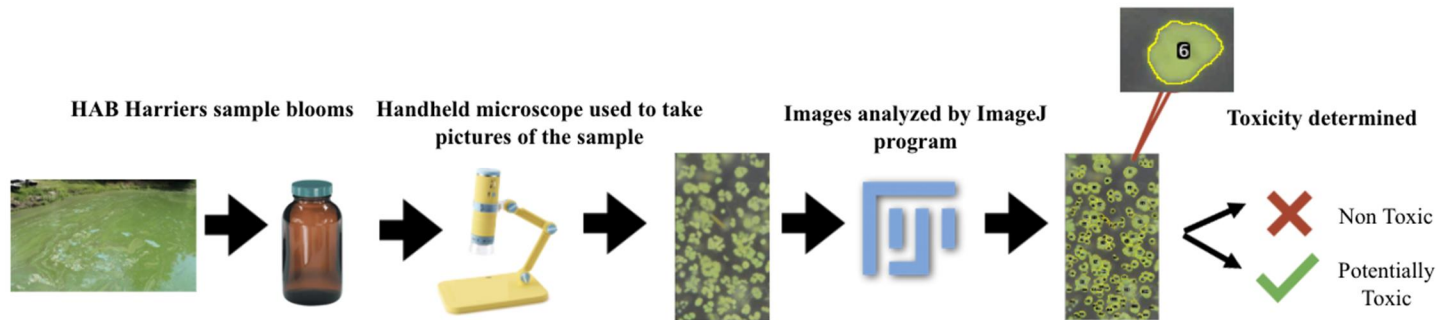
https://docs.google.com/forms/d/e/1FAIpQLSftH8Ia-V5tA3myjaisMMBVd_bxVzLxrM1nrx43JzJJrVDoSg/viewform

Members of the HAB Richardson Lab Team

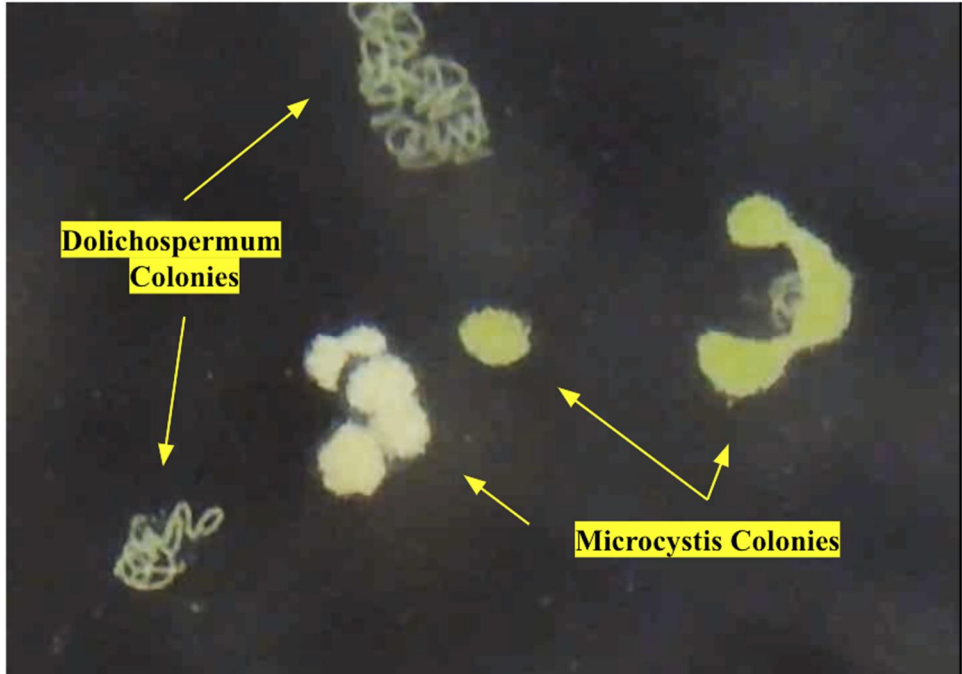
Ruth Richardson; Nan Wang; Kelly Xavier; Chloe Faehndrich; Ilana Hill; Lydia LaGorga; Valerie Aubley

Thanks to our 2020 microscope test project Citizen HABs Harriers monitors: Shelley & Si Meyer, Laura Mirabito, Leo Soderland, Sue Ruoff, David Atwell, and Valerie Aubley.

Potential Images

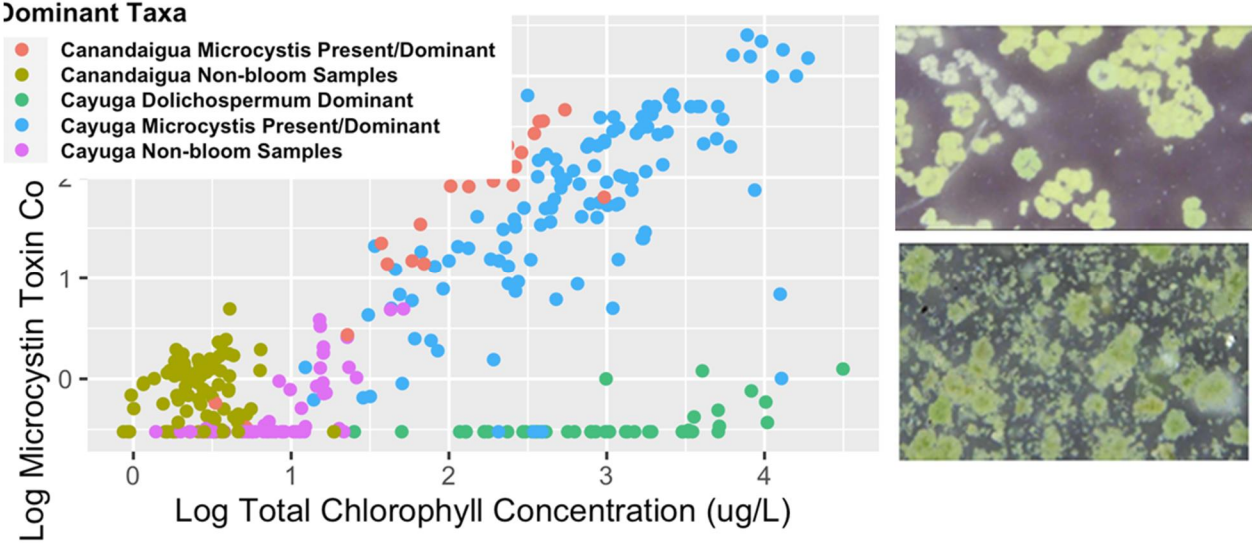


Flow chart Richardson Lab sampling and analysis overview.

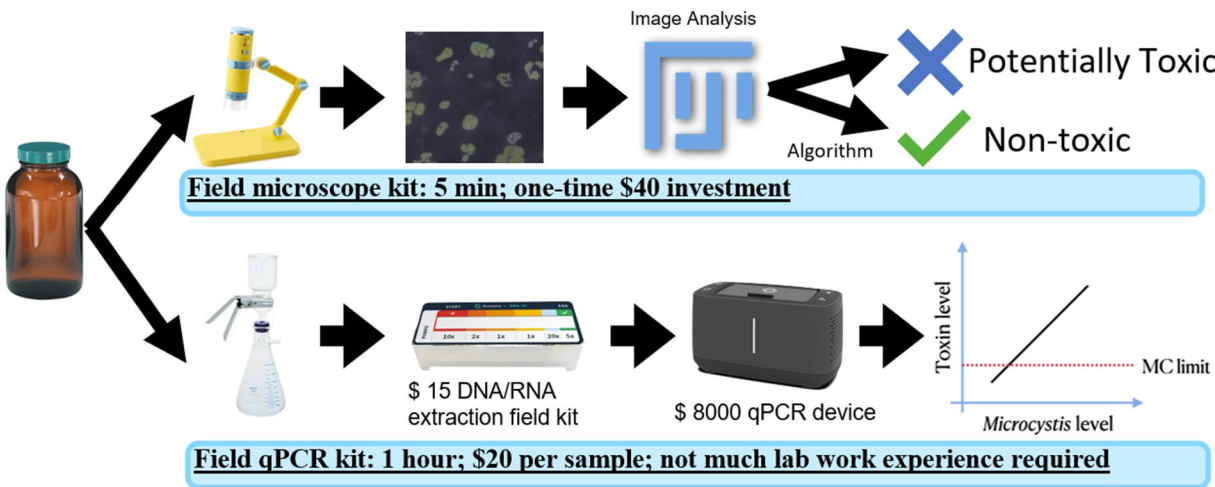


Dolichospermum bloom colonies vs Microcystis bloom colonies. Dolichospermum colonies, usually thin, curly-q shaped, do not produce toxins while Microcystis, which form globular, thick colonies, do release toxins. Sample from zone 3416, Bloom 1, (REF).

OPTIONAL Figures



Workflow of two in-field toolkits tested in 2020



2

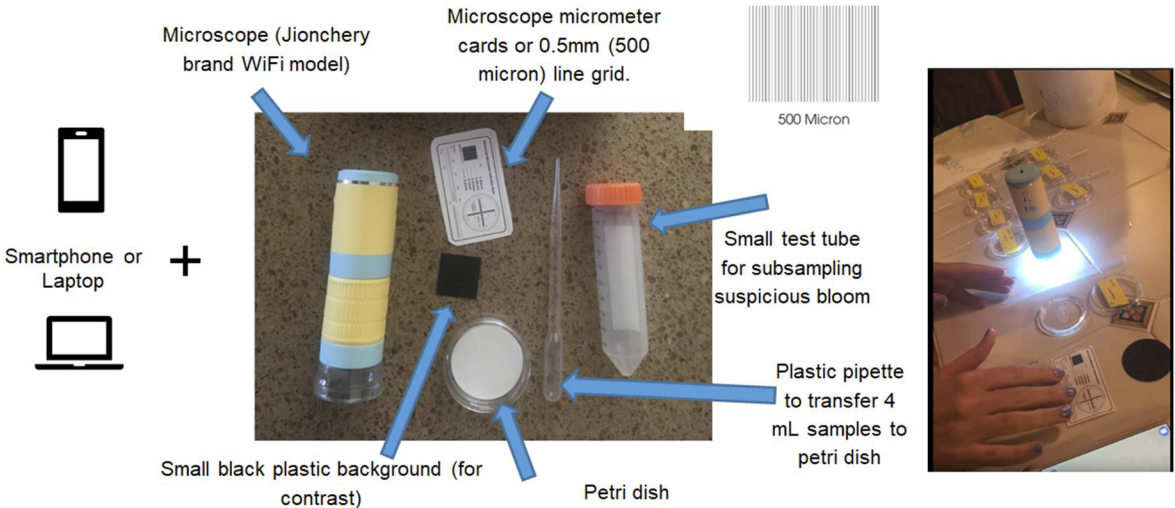
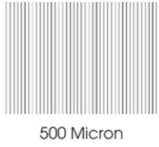


Figure caption: Components of the onsite rapid “cheap” scope kit pilot tested in the Finger Lakes in 2020 (Left). Remote/online training and troubleshooting resources for taking samples and using the microscope were created also, including how to videos (Right)

Photos and videos taken for suspicious bloom



500 Micron

Take a picture and video with 0.5 mm grid as background



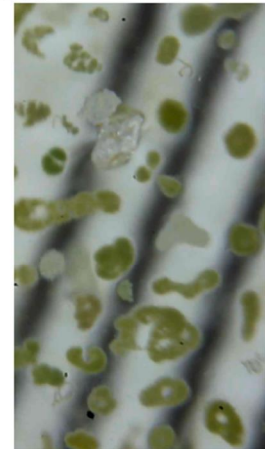
Take a picture and video with black background



3477 7/3/20 (non bloom)



3459 B5 (MC 1.3)
Very sparse Microcystis



3454 "B2" 9/26/20 (MC 303)
Dense Microcystis, dense Dolichospermum, and sparse Oscillatoria

Possible Other Critters to show:

<https://drive.google.com/drive/folders/1pVursMcOfwa0MAMJyaqJgrNhGXpFx71B?usp=sharing>