

Planktoscope: In-situ portable digital imaging instrument

Global HAB Automated in situ sensor symposium

Kristineberg Center, Sweden

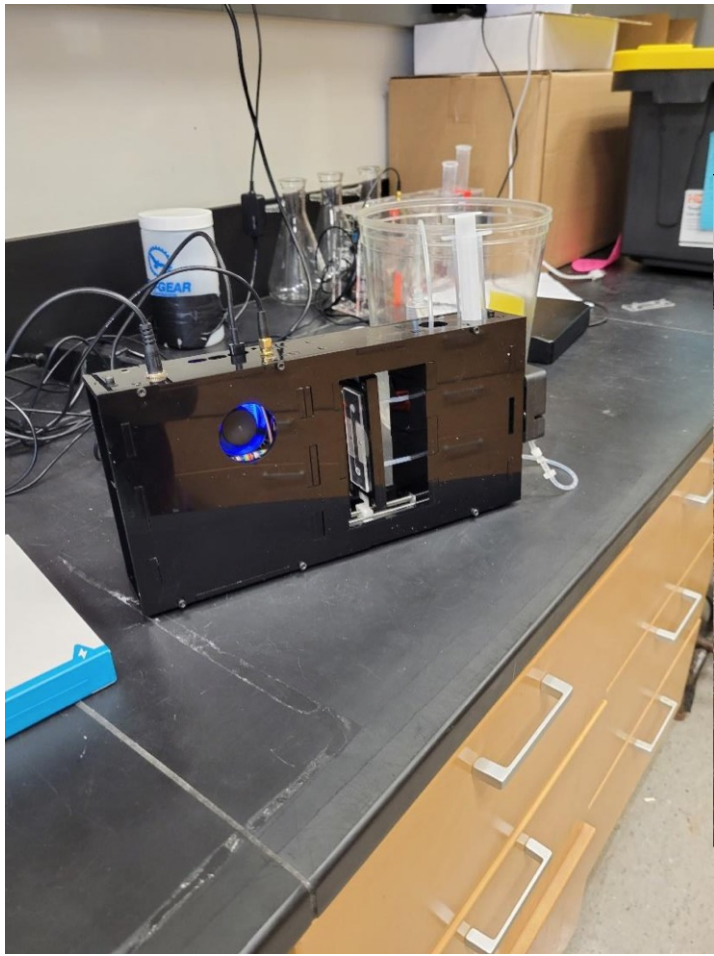
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Clayton Lab planktoscope "Wigg"



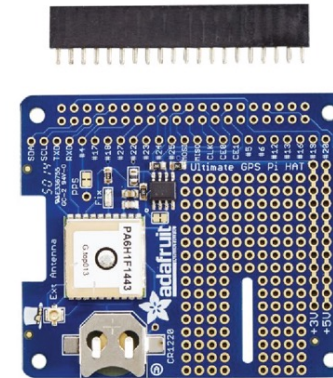
Using the planktoscope
dockside at Norfolk Yacht
Club Study Site (NYCC)



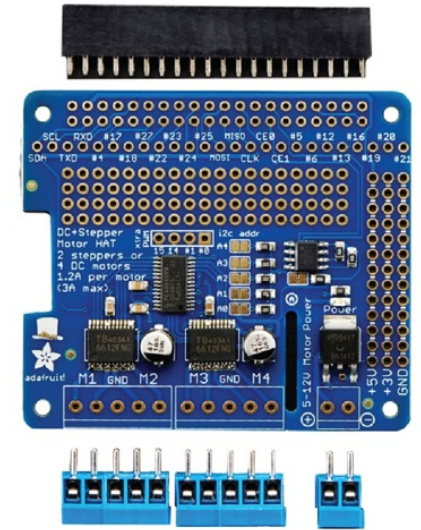
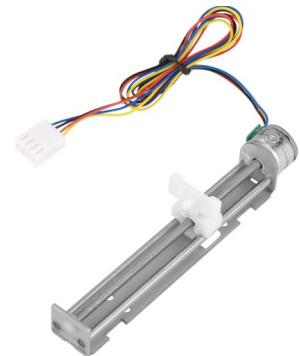
Maci Wigginton

Planktscope Parts List

- **Raspberry Pi 4 B (GB):** single board computer with a 4 GB of memory
- **Adafruit Stepper motor HAT:** this module controls 3 steppers(2 for the focus and 1 for the pump. Stepper motors
- **Adafruit Ultimate GPS HAT:** the module that stores the date and time as well as logs GPS coordinates
- **Yahboom Cooling Fan HAT:** cools and enables visual feedback for functions with LEDs
- **Hammer Header Male 2X 20**
- **Stacking header**
- **Pitch IDC Sockets**
- **Gibbon Ribbon IDC 40P**
- **Power Supply 3A (USB)**
- **Power Supply 1A (USB)**
- **USB Type-C to USB-A 2.0**
- **USB. 5v to DC 12V Step Up**
- **Stepper Motor Peristaltic Pump**
- **Linear Stepper Motor- 2 per unit**
- **Micro SD card + Adapter**
- **Heat Sink Kit for Raspberry Pi**
- **Luer Lock Connectors 1.6 mm (male and female)**
- **Micron slide variety pack (uncoated)**
- **Silicon tubing. 1.6mm**
- **Arducam M12 Lens Kit**
- **Neodymium magnets**
- **LED white 5mm**



Adafruit Ultimate GPS HAT



Cost



- First planktoscope ~\$1,200.00 USD, Second planktoscope ~\$500.00 USD
- All parts are sold on Amazon (sometimes they are on sale during Amazon Prime day)



Benefits and Downfalls

Pros

- Portable and lightweight
- Can image well
- Low cost and part abundance
- Promotes backyard learning & education
- Easy to build
- Higher frequency of sampling in time and space
- Work with samples that non-destructively
- Can handle both live and fixed samples (Lugol's-dilution required)
- Highly customizable

Cons

- Time consuming: takes time to build the classifiers and train the instrument
- Takes pictures and then segments those pictures using Morphocut >1 hr for 5 ml
- only focus image when fluidics is paused
- Acquisition number is not carried through each step from imaging to segmentation and then to download from EcoTaxa
- There is no laser or ROI system in place (area of interest problems)
- Tubing gets clogged (reverse the direction of tubing so that shortest distance from samples to flowcell)
- Cell overlapping in images –manually count
- Underestimates counts ~40% of the time



Process

1. Calculate the frame rate depending on how much volume you are sampling
2. Run test runs to determine reasonable focus
3. Run sample
4. Run segmentation process
5. Export images and data to EcoTaxa
6. Validate the images, re-classify the images if classified wrong
7. Write code (Python/MATLAB) in order to extract the data of the cells

~39 lugol's samples of 5 ml each, took about 2 months with proper taxonomic identification



Ecotaxa

- Web based application
- It has a built in reference taxonomy and you can build a taxonomy tree for training
- Images can be directly uploaded to ecotaxa after the segmentation process
- Validate certain images of organisms
- Once validated the images you can hit the retrain your instrument and permit to predict the identification through automatic classification

The screenshot displays the Ecotaxa web application interface. At the top, the project name is "Planktoscope-OEAS-ODU (15765, 0, 0, 0 / 15765)". The user is identified as "Jacqueline Chrobot (log out)" with a status of "Error :1 Done :2". A filter is applied: "Taxo= Cochlodinium 02".

The left sidebar shows a taxonomy tree with the following categories and counts:

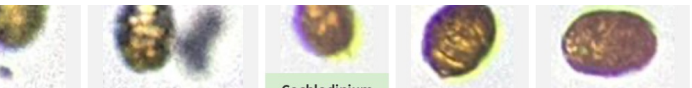
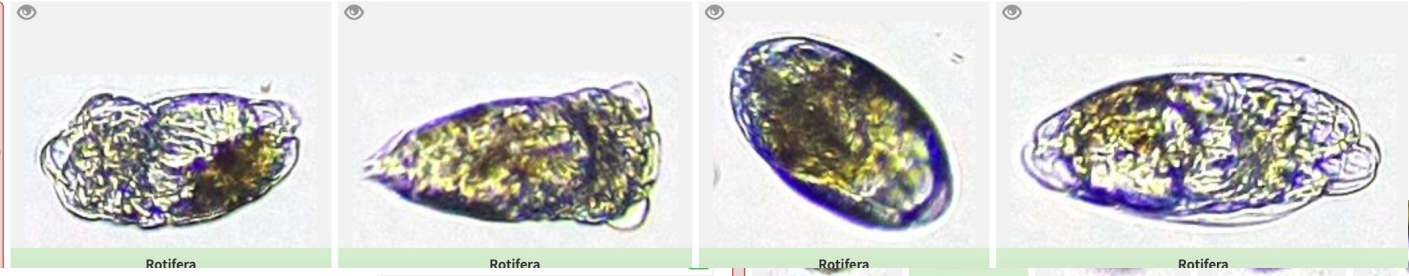
Category	Count
Cochlodinium	2102
Cochlodinium polykrikos	1650
Cochlodinium sp.	1227
Cochlodinium 01	8
Cochlodinium 02	1
Diatoms	1
Dinoflagellates	4
Ditylum	2
Ebria tripartita	1
Eucampia	114
Heterocapsa	1
Melosira	2
Nematoda	1
Polykrikos sp.	4
Proboscia alata < Proboscia	2
Prorocentrum < Prorocentrum	1
Pyramimonas	6
Rhizosolenia	1

The central menu includes the following options:

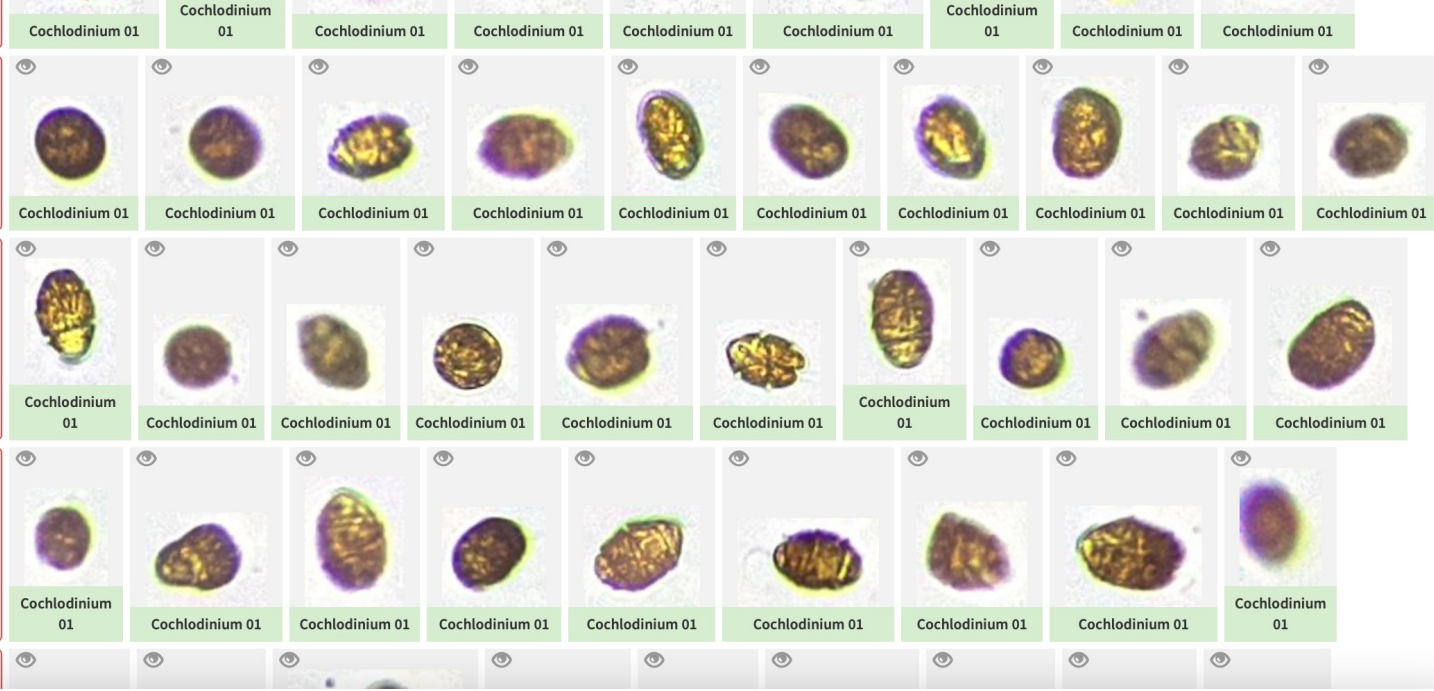
- Train and Predict classifications
- Import images and metadata
- Fix category issues
- Export
- Edit project settings
- Extract Subset
- Merge another project in this project
- Edit or erase annotations massively
- Batch edit metadata
- Reset status to Predicted
- Delete objects or project

The main area shows a grid of 24 images of organisms, each labeled "Cochlodinium 02". The interface includes various controls such as "Update view & refresh", "Display", "Status", "All", "100", "100%", and "Action".

Cochlodinium 01	1433
Cochlodinium 02	15765
Diatoms	21949 24777 5
Dinoflagellates	1650 3102
Ditylum	1227 8 1
Ebria tripartita	2
Eucampia	1
Heterocapsa	114
Melosira	1
Nematoda	4
Polykrikos sp.	2
Proboscia alata < Proboscia	1
Prorocentrum < Prorocentrum	1
Pyramimonas	6
Rhizosolenia	8
Rotifera	1952 11 12
Staurastrum	1
artefact	2316
badfocus < artefact	163595



Pleurosigma	460	2	
Pseudo-nitzschia	4		
Thalassionema	270		
Chaetoceros sp.	6934 9895		
Ciliophora	700	5	88
▼ Cochlodinium	14427	2	
Cochlodinium polykrikoides			
Cochlodinium	0		
Cochlodinium sp.	428	5	
Cochlodinium 01	1433		
Cochlodinium 02	15765		
Diatoms	21949 24777 5		
Dinoflagellates	1650 3102		
Ditylum	1227 8 1		
Ebria tripartita	2		
Eucampia	1		
Heterocapsa	114		
Melosira	1		
Nematoda	4		

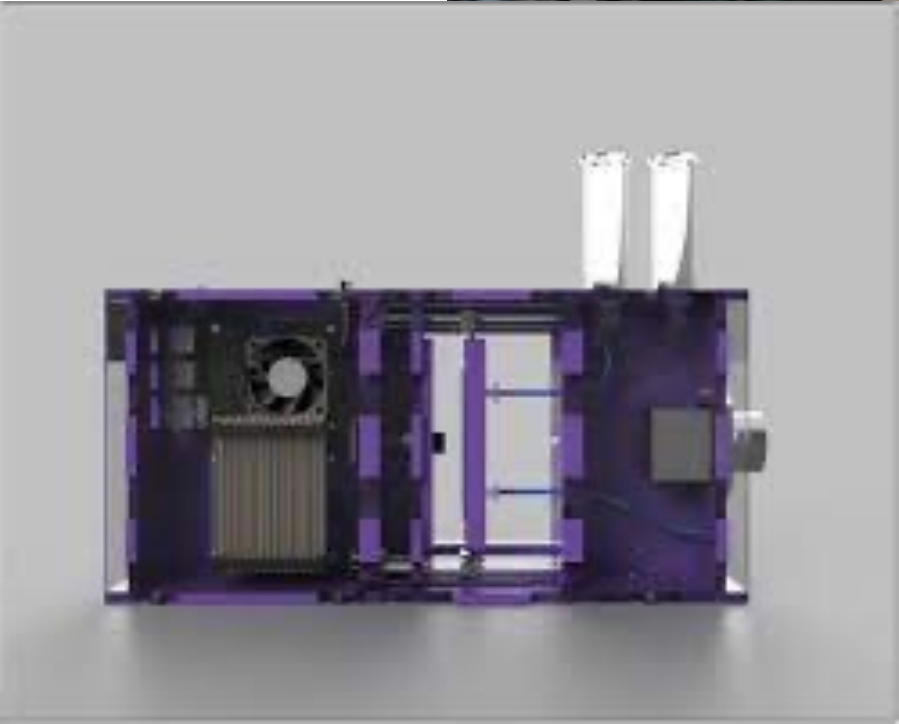


Best practices of EcoTaxa

- All planktoscopes can be linked to the same project (sharing of the classifier/ pictures) OR
- You can share images across different projects, however, you will have to validate all images in all different projects
- ~200,000 images does a fairly descent job
- Use a different more durable planktoscope exterior casing
- Use specific acquisition ID's to track your sample through each planktoscope process
- Date and time should be when sample was taken, not when it is analyzed
- Clean flow cell
- Acetone/alcohol to clean tubing regularly
- run the planktoscope backward, create a smaller distance between sample and flow cell



Version 4 Planktoscope



References

Clayton, S., Gibala-Smith, L., Mogatas, K., Flores-Vargas, C., Marciniak, K., Wigginton, M., & Mulholland, M. R. (2022). Imaging Technologies Build Capacity and Accessibility in Phytoplankton Species Identification Expertise for Research and Monitoring: Lessons Learned During the COVID-19 Pandemic. *Frontiers in Microbiology*, 13.

Irison J.-O., Ayata S.-D., Lindsay D. J., Karp-Boss L., Stemmann L. (2022). Machine Learning for the Study of Plankton and Marine Snow From Images. *Annu. Rev. Mar. Sci.* 14, 277–301.

Mériguet, Z., Oddone, A., Le Guen, D., Pollina, T., Bazile, R., Moulin, C., ... & Lombard, F. (2022). Basin-scale underway quantitative survey of surface microplankton using affordable collection and imaging tools deployed from Tara. *Frontiers in Marine Science*, 1125.

planktoscope.slack.com

<https://www.planktoscope.org/replicate/get-your-kit>

<https://github.com/PlanktoScope/PlanktoScope>

Thank you!
Questions?

