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Planktoscope protocol for plankton imaging

Fabien Lombard

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Planktoscope protocol for plankton imaging V. (bytypwpw)

Lombard Fabien

bytypwpw

1

L Lombard Fabien

this protocols applies to the version 2.1 to 2.3 of the planktoscope and the 2.3 version of software. it is optimised to image 40µm-200µm organisms using the 25mm lens (as tube lens) and 16mm one as objective one and may be inaccurate with other configurations or light. Please note that the segmenter in currently also optimised for this and may need to be recoded (or adjusted) for other configurations, notably the size threshold but also the intensity threshold

this protocol is for using planktoscope and collect usable result for quantitative imaging of plankton

see also <https://www.planktoscope.org/>

Pollina et al 2020
2020.04.23.056978v1.full.
pdf

<https://www.planktoscope.org/>

Lombard Fabien . Planktoscope protocol for plankton imaging. **protocols.io**
<https://protocols.io/view/planktoscope-protocol-for-plankton-imaging-bytypwpw>

protocol

Pollina T, Larson A, Lombard F, Li H, Colin S, Vargas C de, Prakash M (2020)
PlanktonScope: Affordable modular imaging platform for citizen oceanography. bioRxiv
2020.04.23.056978. doi: 10.1101/2020.04.23.056978

Planktoscope, plankton

Fabien Lombard, Thibaut Pollina

Oct 06, 2021

Jan 04, 2022

53848

Planktoscope is an optical instrument. As its optical elements (camera, lenses, flowcell) are highly sensitive to dust and dirt, we recommend that you never touch any of those components with fingers and store the planktoscope in a dust-free and humidity-free area (or in a box when not used)

Complete manual of assembly and software could be found at <https://planktoscope.readthedocs.io/en/latest/>

Plankton net
200µm sieve
Squeezing bottle
micrometer slide (or millimetric ruler)
Optical paper
Dry gas dispenser

Planktoscope is an electronic device, powered with electricity. It is therefore sensitive to water.

- Place it in an environment where water can not enter in contact with the instrument and secure its electrical part.
- Be careful when manipulating samples, take care of having the exhaust tube in a "trash" container to avoid spillage
- Glass parts are present (flowcell) and should be manipulated with caution (can break and injure you), but also should be kept clean (avoid touching it with fingers)

:

This protocol applies to the version 2.1 to 2.3 of the planktoscope and the 2.3 version of software. It is optimised to image 40µm-200µm organisms using the 25mm lens (as tube lens) and 16mm one as objective one and may be inaccurate with other configurations or light. Please note that the segmenter is currently also optimised for this and may need to be recoded (or adjusted) for other configurations, notably the size threshold but also the intensity threshold

- Test the protocol before acquisition of your first sample
- Calibrate your instruments to ensure coherent measures
- Create an Ecotaxa account and request the right to create project way before
- Collect a plankton sample using a net

first use and basic check

- 1 Make sure the different lenses are in place (tube lens on the camera side is a 25mm/ objective lens on the light side is a 16mm one). Insert the Ibidi flowcell on the flowcell stage. Assemble the fluidic part



tube / objective lenses in place. Ibidi flowcell on its stage



assemble the fluidic parts



put everything in place



- Try to get the shortest path as possible between the sample syringe and the flowcell
- Try to not get horizontal path between the syringe and the flowcell
- Orient the flowcell in order that it flows vertically from up to down

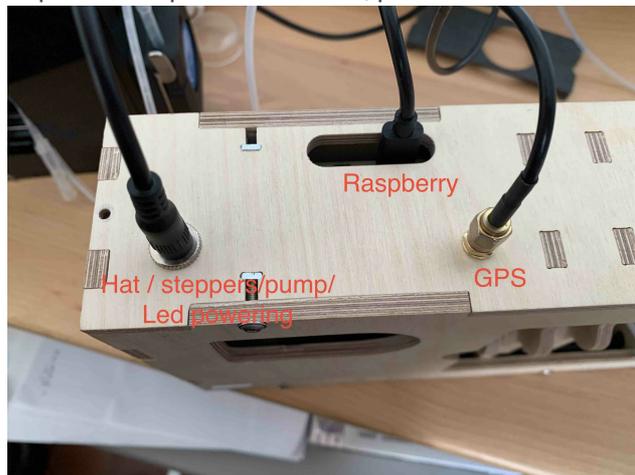
If not followed properly, plankton have the tendency to sediment and will progressively creates form clogs in the fluidic system which may severely lead to undersampling of plankton

- Choose tubing which is the smallest as possible (eg. if flowcell is 200 μ m wide, tubing with internal dimensions >1mm is not needed and will creates larger stagnation of the plankton, larger risks of sinking in the fluidic path and also larger chance that plankton can swim against the current)



example of optimised setup to avoid long path between sample and flowcell

- 1.1 power you planktoscope by connecting the different cables if the usb-c cable powering the planktoscope have a switch, put it on.



- 1.2 wait few minutes and you should see a wifi appearing with the name "planktoscope_baba...."
Connect to it (password "copepode")

it is possible to connect by ethernet instead if you do have a cable

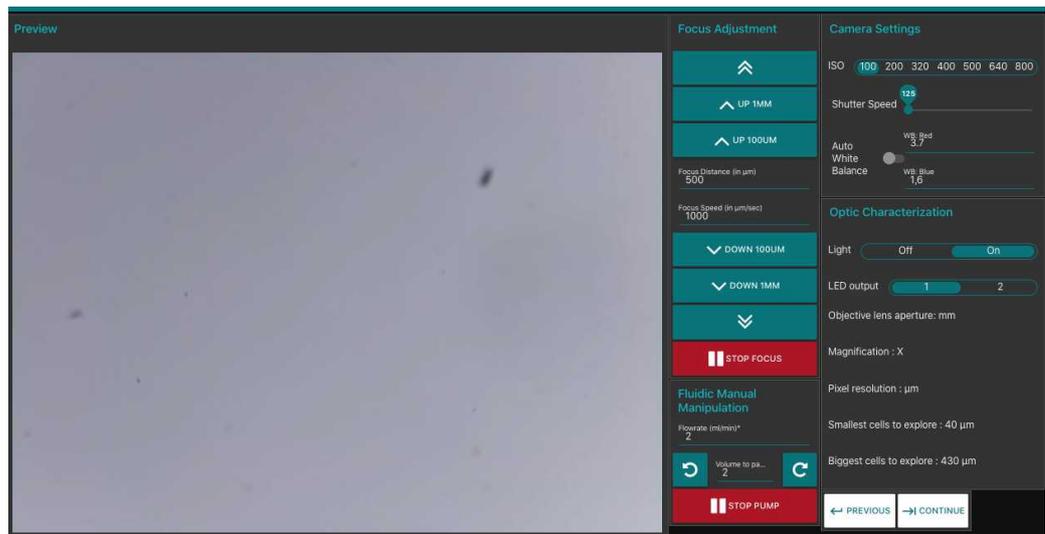
- 1.3 connect to the planktoscope using the following webpage
<https://planktoscope.local:1880/ui>
 alternative url <http://192.168.4.1:1880/ui>

 For software versions previous to 2.3

- For windows users, you may need to download the "bonjour" printing system to communicate with the planktoscope: see here : <https://support.apple.com/kb/d1999>
- For mac users, this shouldn't be a problem
- for linux, we don't have tested it and may not have a solution

alternative url to debug (no camera return) <http://192.168.4.1:1880/ui/>

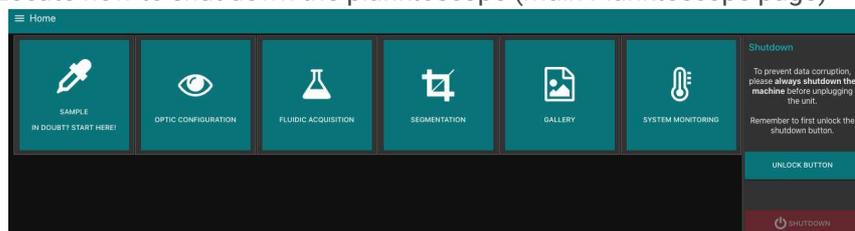
- 1.4 Check that your planktoscope is properly operating: on the optic configuration page:
- turn the light on/off (you should see the light and the camera overview got white)
 - check that adjusting the focus makes the focus motor to turn
 - check that the pump motor is functioning by pumping a little
 - check that changing ISO/ white balance/ shutter speed leads to changes on the camera



optical configuration window

- 1.5 adjust manually the white balance in order that it looks about similar to the automatic white balance (AWB) this latter should be desactivated once you obtain correct results (it will continue to correct depending on the images and may leads to incorrect results)

- 1.6 Locate how to shut down the planktoscope (main Planktoscope page)

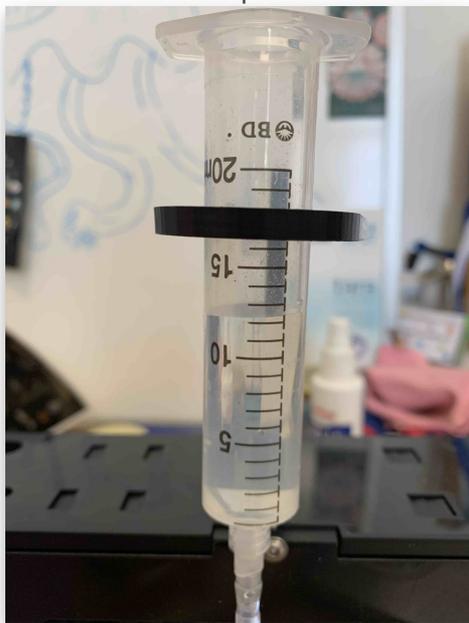


2 Pump calibration:



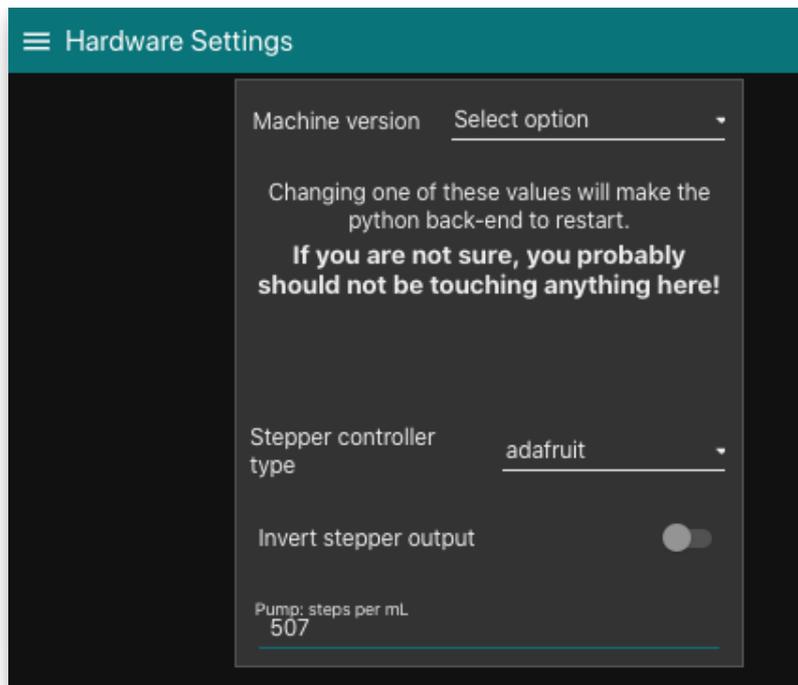
Peristaltic pump tubes flexibility varies with age, care and type of liquid used (e.g. lugol may age it quicker), calibrating the pump regularly could be needed but is not highly important to get good quantitative count since this is the number of image (therefore the volume imaged) which is important (not the pumped volume)

- 2.1 -prepare a large volume of tap water and put in in the syringe targeting a total volume of e.g. 20ml
-on the optic configuration tab: tell him that you want to pass 10ml and record the exact volume it finally ends to pass (eg. by looking on the graduation of the syringe)
note X= final volume passed for a 10ml instruction



final volume (here $X=20-12.2$)

- 2.2 -then on the hardware setting, note the "pump step per ml" parameter (old step)
-calculate the "calibrated" pump step per ml such as $= 10 \cdot \text{old step} / X$
-replace the "pump step per ml" parameter with this value



hardware settings page (pump per step is at the bottom)

3 Size calibration:



Size calibration is an important process to get good data and should be absolutely done and noted. Please however note that currently calibrations is currently bugged and you need to **manually replace the comma by a point in the hardware setting at every start**

3.1 - tilt the planktoscope on the side (camera on the bottom)

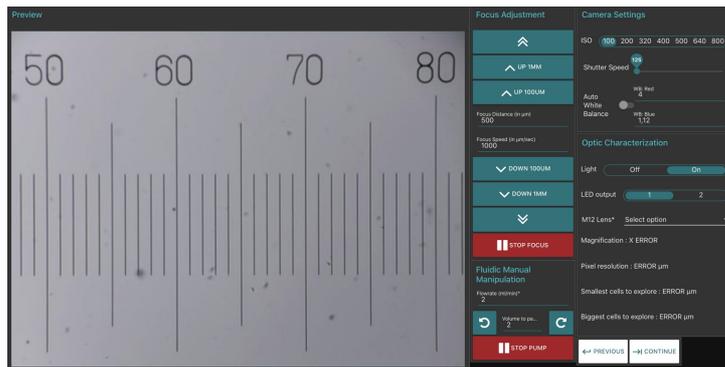


tilted position for calibration

- 3.2 - remove the flowcell and place a micro metric ruler (or a millimetric one) on the sample stage such as the ruler is either vertical or horizontal **but not in diagonal** (using the 20mm/16mm combo of lenses the camera field of view should be about 3mm by 4mm). Make the focus on the scale

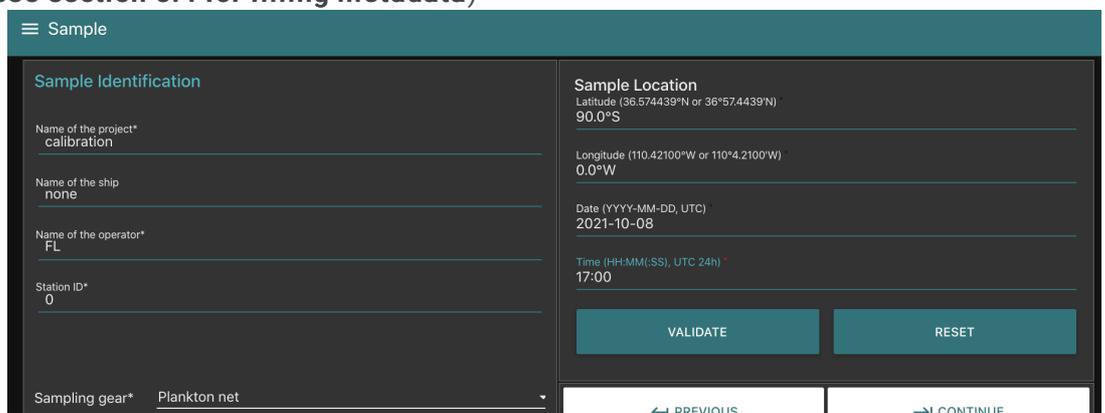


placing the calibration slide (once removing the flowcell)

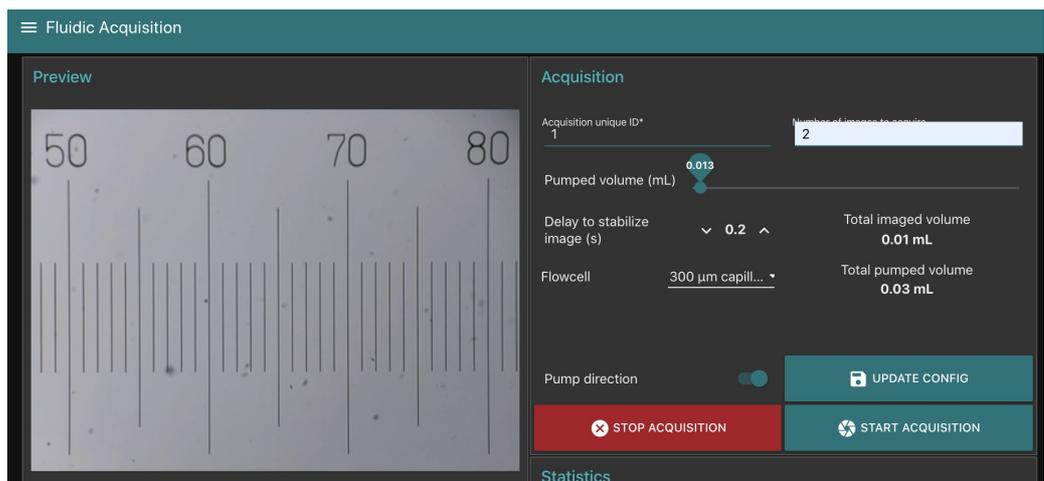


focusing on the scale

3.3 -take few images (enter sample name, positions don't matters but enter 90S, 0W to easily spot the sample as a test, date and time, these are the only mandatory fields; see section 5.4 for filling metadata)

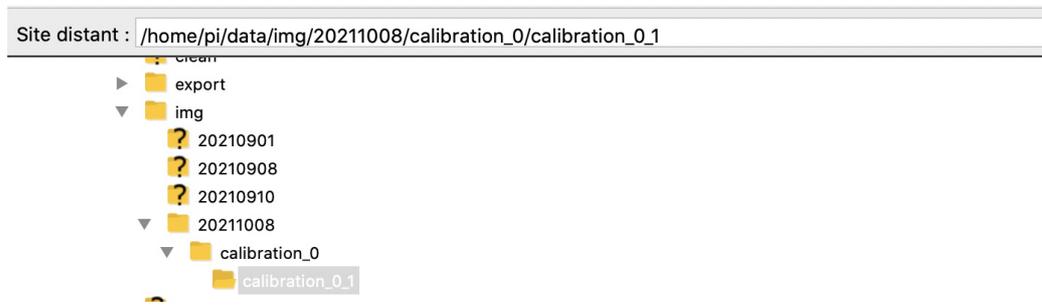


example of metadata entered in "sample" page

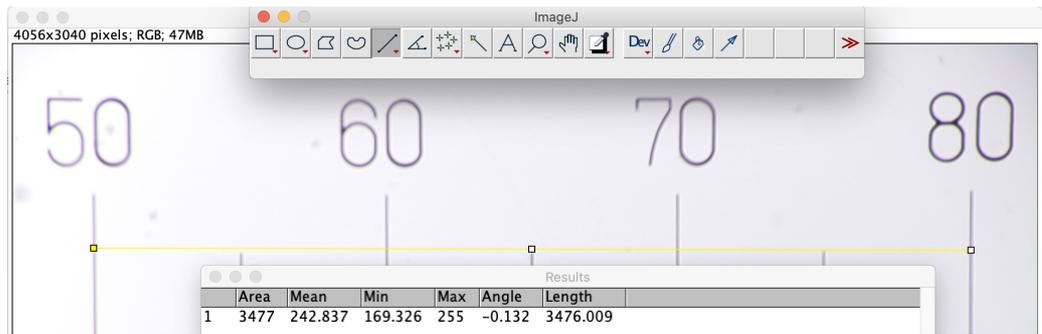


here two images are acquired (and random information entered on the pumping one)

3.4 -download images on a computer and measure how much pixels are needed to obtain the longest path possible on the image (e.g. using imageJ <https://imagej.nih.gov/ij/index.html>) (see section 7 for communicating with your planktoscope and downloading the raw images)

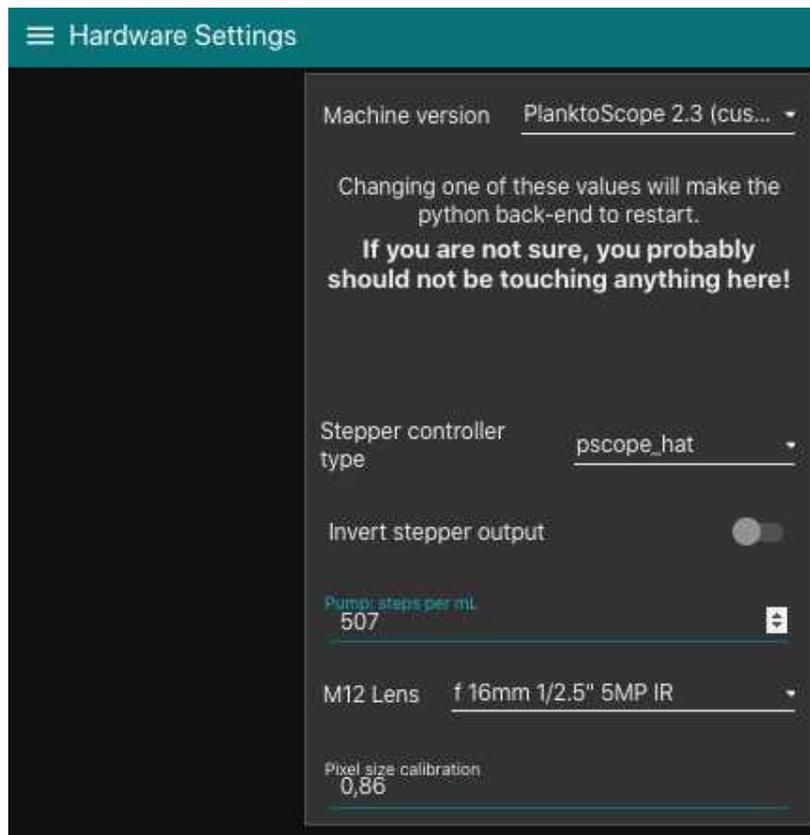


downloading resulting images (using Filezilla)



using imageJ open your image (File/open), draw a line as long as possible (here 3mm on the scale) and measure it (analyse/measure). The line is 3476 pixel length (i.e. one pixel is 0.86 with this example)

- 3.5 -calculate how much micron is represented by each pixels (should not be strongly different from 1.01 which is the default value for 25/16mm lenses combo)
- 3.6 Enter the calibrated pixel size value in the hardware setting (**note here that the comma should be replaced by point at every restart of the system**)



Get your sample

4 Use a net to collect plankton



Record **Latitude Longitude** (taking photo of the GPS when launching and recovering the net could serve, if UTC time could be present on the GPS this could also be interesting)
-if **vertical net**, record **min and max depth**
-if horizontal records **initial/final positions, speed and length (min) of deployment**
-if you have **flowmeter**, record the **initial/final digits of the flowmeter** and calculate the **filtered volume**

in all cases the **diameter of the net opening** will be needed

Those are critical informations to get to quantitative sampling (see step 5.4)

4.1 Get the content of the collector.

4.2

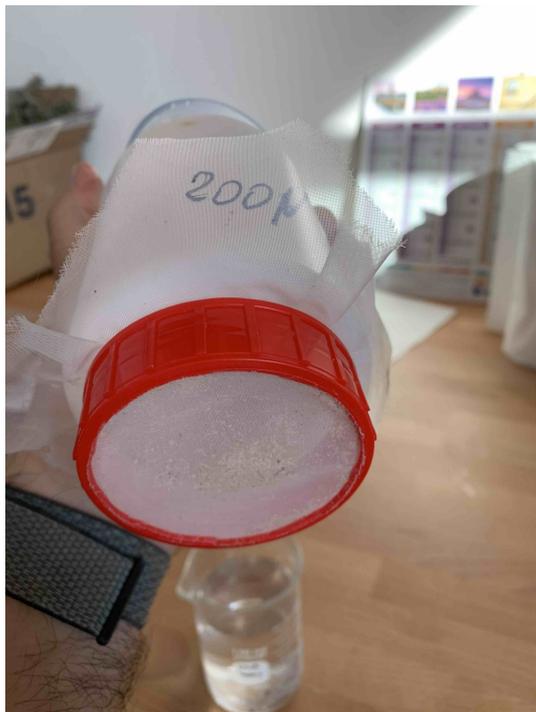
Pass the volume through a 200µm sieve



larger organisms may clog the flowcell



filtration through a 200µm mesh

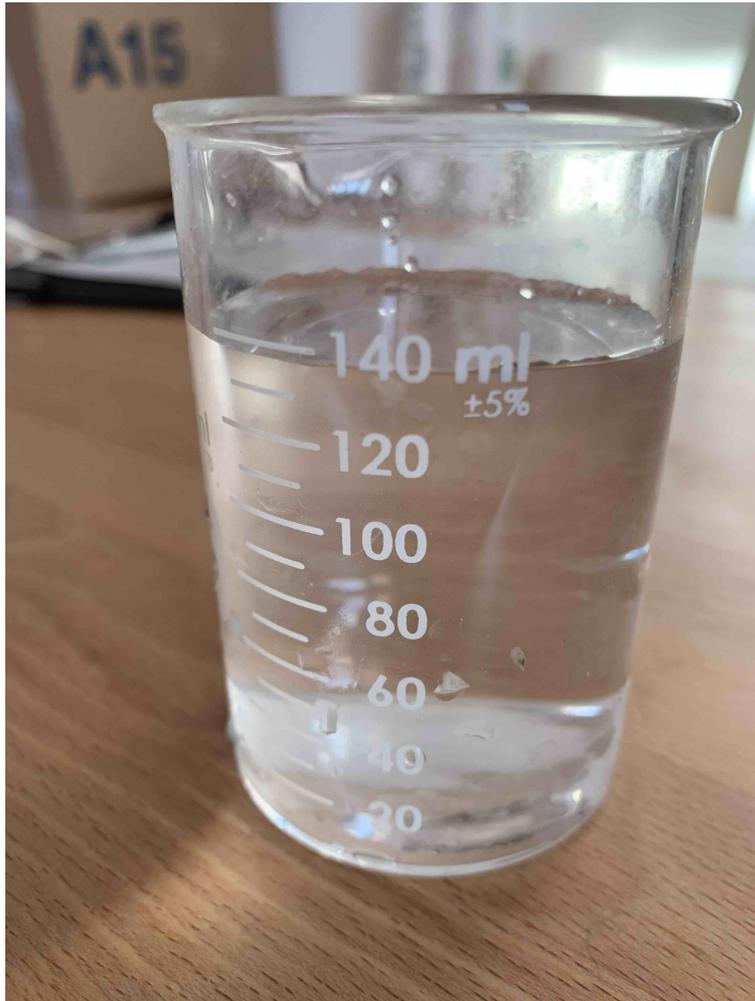


detail of the (home made) filter

- 4.3 Rinse the sieve using seawater and a squeezing bottle (helps to pass small object)
- 4.4 Recover the fluid / measure it volume/ record it on logsheets (will be entered latter as **"concentrated sample volume"**)



Those are critical informations to get to quantitative sampling



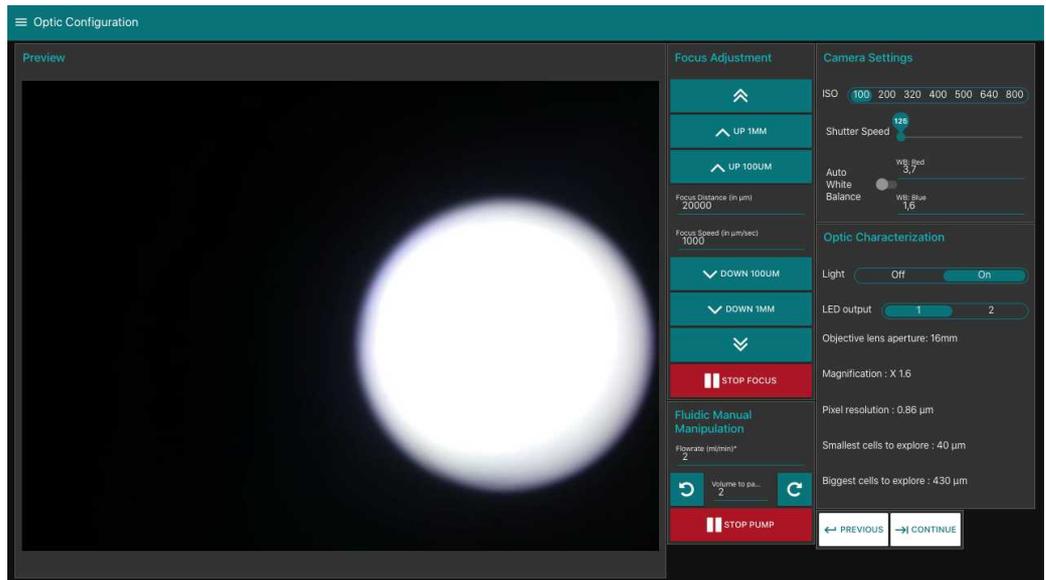
concentrated final volume of plankton

Pass the sample on planktoscope

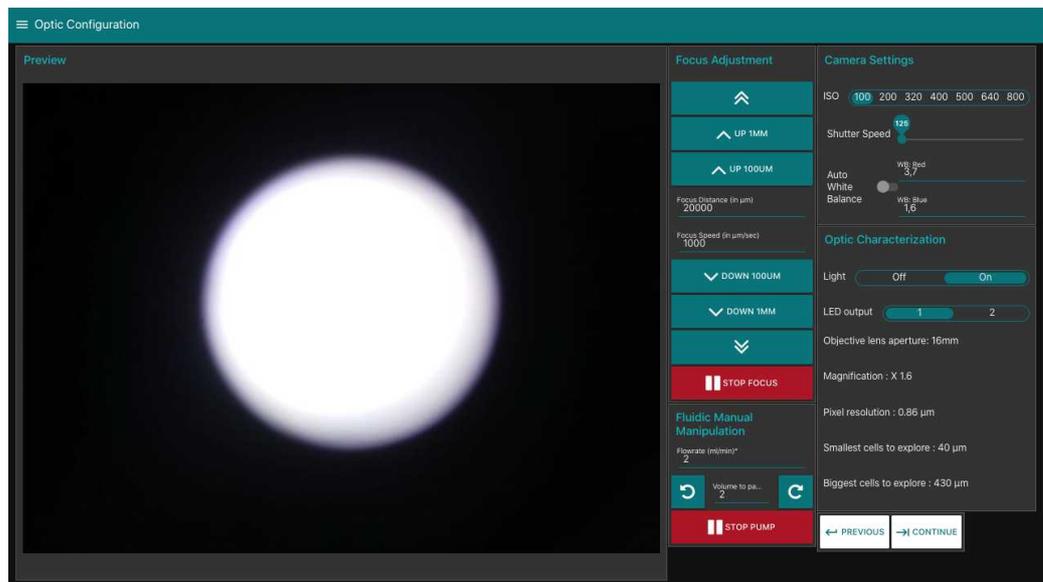
- 5 assemble and start the planktoscope (see [go to step #1](#) [go to step #1.1](#) [go to step #1.2](#) [go to step #1.3](#))
 - 5.1 check for lenses alignment : remove the objective lens, start the light and check if the light source is centred.
 - if yes place back the objective lens and flowcell
 - if no adjust the position of the tube lens to center the light source (magnets allows for 1-2mm adjustments)



objective lens and flowcell removed

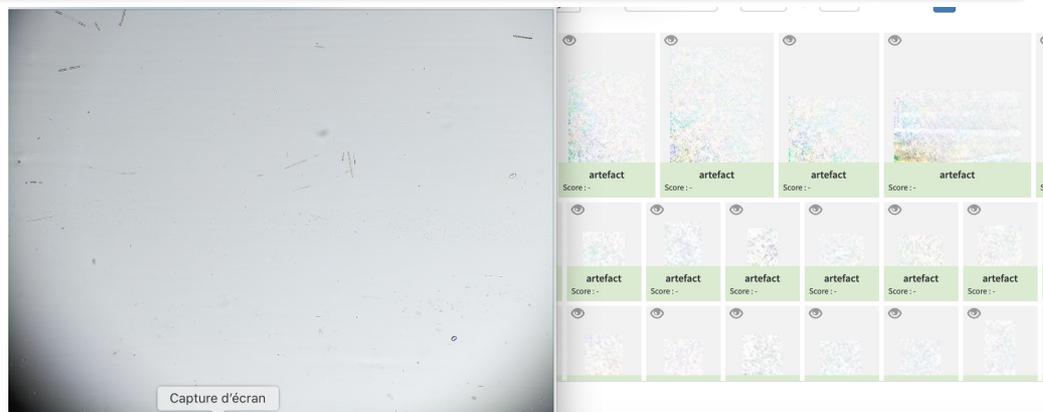


unaligned lens



centered lens

⚠ unaligned tube lens could create strong inhomogeneous background in final images, leading to creating lots of artefact during segmentation of the different plankton objects. Unfortunately the magnets lets a 1-2 mm degree of freedom which is responsible from this



severely unaligned lens final results and and final artefact object created

5.2 Go to optic configuration.

- turn the light on
- verify focus on dry slide (tip, if the slide is slightly wet, you can check for the focus simultaneously on the water traces on the two sides of the flowcell)
- Add your sample in the syringe (and keep it suspended by agitating it manually regularly or even gently insert an air bubbler with 1 bubble/second in it)
- pump until you see your sample passing by and flowing through the peristaltic pump
- Eventually get rid of air bubble by pinching the tubing half way between the flowcell and the pump while pumping (see also 5.7).
- finely adjust the focus on the organisms passing by (tip: start using the "1mm" buttons, then the 100µm buttons and finalise by typing 25 or 50µm adjustments in

the middle box (and pressing external arrows of focus)



not agitating your sample will let plankton to sediment and could even block the fluidic part. More importantly, the organisms concentration will be inhomogeneous, and because you will first get the sinking plankton, will lead your measurements to over-estimate true concentrations

- 5.3 adjust the concentration of the sample: **ideally not more than 20-30 objects** should be present per frame. If the sample is over-concentrated, dilute it by a factor 2 (add in a jar 1/2 of the sample -after agitating it- and 1/2 of seawater). **Note dilution xxx in metadata in 5.4**



Having too much object per frame will create :

1. increase probability that objects are touching (making them impossible to count or identify)
2. increased probability to clog the fluidic system
3. artefacts during the segmentation step

- 5.4 Go to "sample" page and fill metadata

This step is critical because those data are the ones that will make your sample usable or not

-**fill the sample identification** (project, name, boat used, your name and the station number)

Sample Identification

Name of the project*
Tests

Name of the ship
Kayak

Name of the operator*
Fabien Lombard

Station ID*
1

-**tells how you sampled the plankton** (recording mesh size with "**minimal fraction size**" (will be used afterwards in the segmentation process, object smaller than this wont be segmented; "**Maximal fraction size**" is the size of the mesh used in step [↩ go to step #4.2](#) ; **Filtered** volume is important if you recorded it but could be calculated from other parameters . Make sure to either have filled it or to have filled either **min and max depth** if using a vertical net; **initial/final positions, speed and length (min) of deployment** if using an horizontal towed net

in all cases the **diameter of the net opening** will be needed to calculate the filtered volume

Dilution factor if dilution have been done in [go to step #5.3](#)

Sampling gear* Plankton net

Minimal fraction size (µm) 20 Min sampling depth (m) 0

Maximal fraction size (µm) 200 Max sampling depth (m) 2

Filtered volume (in L)

Concentrated sample volume (mL) 138

Dilution Factor

Speed Through Water

Net opening dimension (mm) 300

Fill the net initial and final position (if towed horizontally) remember to validated both of them (readings disappear after validation, but are recorded)

Net Throw Location
Latitude (36.574439°N or 36°57.4439'N) 43.696369°N
Longitude (110.42100°W or 110°4.2100'W) 7.307533°E
Date (YYYY-MM-DD UTC) 2021-10-15
Time (HH:MM, UTC 24h) 11:30
VALIDATE RESET

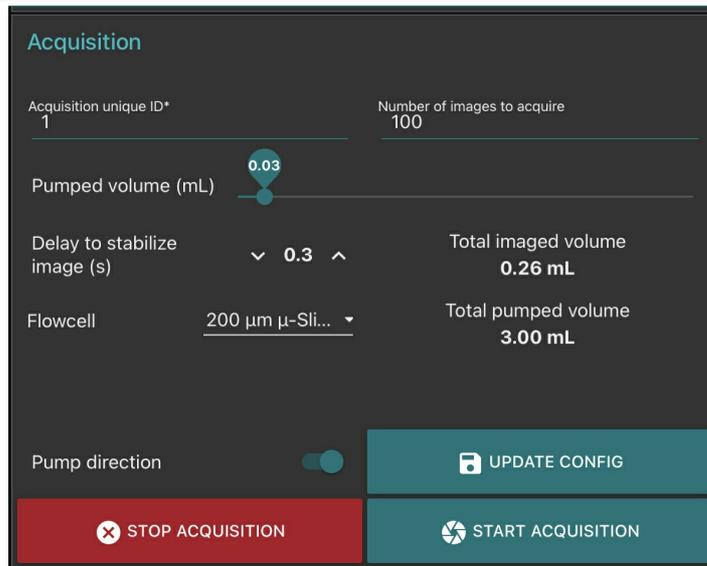
Net Retrieval Location
Latitude (36.574439°N or 36°57.4439'N) 43.696152°N
Longitude (110.42100°W or 110°4.2100'W) 7.307967°E
Date (YYYY-MM-DD UTC) 2021-10-15
Time (HH:MM, UTC 24h) 11:40

5.5 Go to fluidic acquisition and set parameters
-number of image to acquire (to be chosen depending on the desired final object number and the observed concentration on images)

 pump significantly between two images will help to :

1. not let plankton to sediment in the fluidic system
2. not image two time the same plankton

Target a sample size (by setting the number of images to acquire) that finally have something like 1000-2000 final objects (e.g. if you do have 10 object per images, imaging 100-200 frames would be enough)



5.6 go to optic configuration and pump with high flow rate a good amount of water (objective, remove plankton that have sunk in the fluidic system)



5.7 

Go to fluidic acquisition and start the acquisition.
 Awaits that the acquisition is done
 Results could be consulted by passing through the gallery img

 If your fluidic system is not optimised to avoid plankton sedimentation, some plankton could accumulate in the fluidic system. This could be check by pinching on the tube half way in between the flowcell and the pump during 1-2 seconds (to accumulate suction pressure) and releasing it. If a large quantity of plankton pass suddenly then plankton have sedimented between the syringe and the flowcell.



segment the acquisition

6

- Go on segmentation and clic on the "update acquisition's folder list"
 - select the samples you wish to segment
 - Setup the different options of the segmenter
1. Recursive folder means that it will segment all samples within a selected sample
 2. Ecotaxa archive: it will create a zip file containing all files needed for a easy importation within ecotaxa
 3. Force rework: if yes it will re-segment samples already segmented
 4. Keep objects: it will keeps the final segmented images visible in the planktoscope (that could be accessed by the gallery in the objects folder)

Control

You can choose here in which folder(s) you want the segmentation script to run. A few details though:
The segmentation is run recursively in all folders. So if you select a top level folder, the segmentation will be run in all subfolders.
Also, you will be able to chose wether for force the segmentation for folders in which it has run already.

Recursive folder No Yes Force rework No Yes

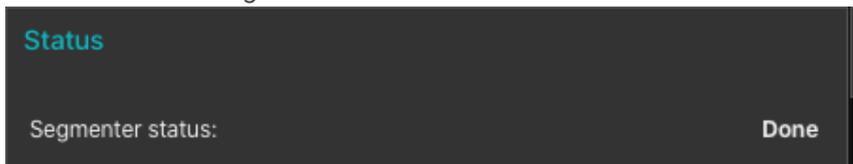
Ecotaxa archive No Yes Keep objects No Yes

 UPDATE ACQUISITION'S FOLDER LIST

20210901

- scroll down and clic on start segmentation

- awaits that the segmenter status turn to "done"



download the results



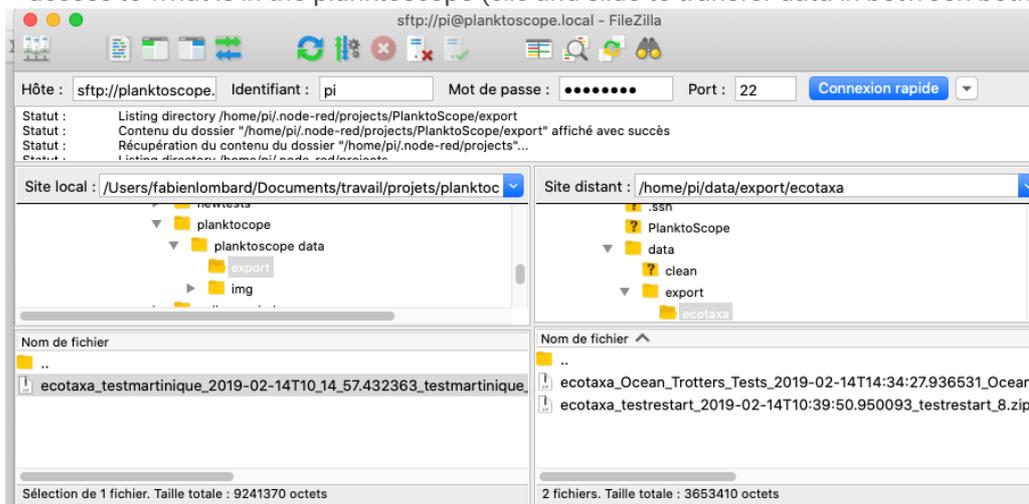
You will need a computer connected to the planktoscope together with free software FileZilla (<https://filezilla-project.org/>)

- Open FileZilla
- either clic on the top right create a new connection or use the quick-connection fields bellow

- Enter the following informations:
 Host: sftp://planktoscope.local (192.168.4.1 should also works)
 Username: pi
 Password: copepode
 Port: 22



- clic on connect
- on the bottom panels you have (at left) the access to what is in your computer and (at right) the access to what is in the planktoscope (clic and slide to transfer data in between both)



Exports file for EcoTaxa are in /home/pi/data/export/ecotaxa
 Raw images files are in /home/pi/data/img
 Different control files to check the segmentation process (images after background substraction, masks of the different objects etc) are in /home/pi/data/clean
 Final vignettes are in /home/pi/data/objects

Clean the planktoscope

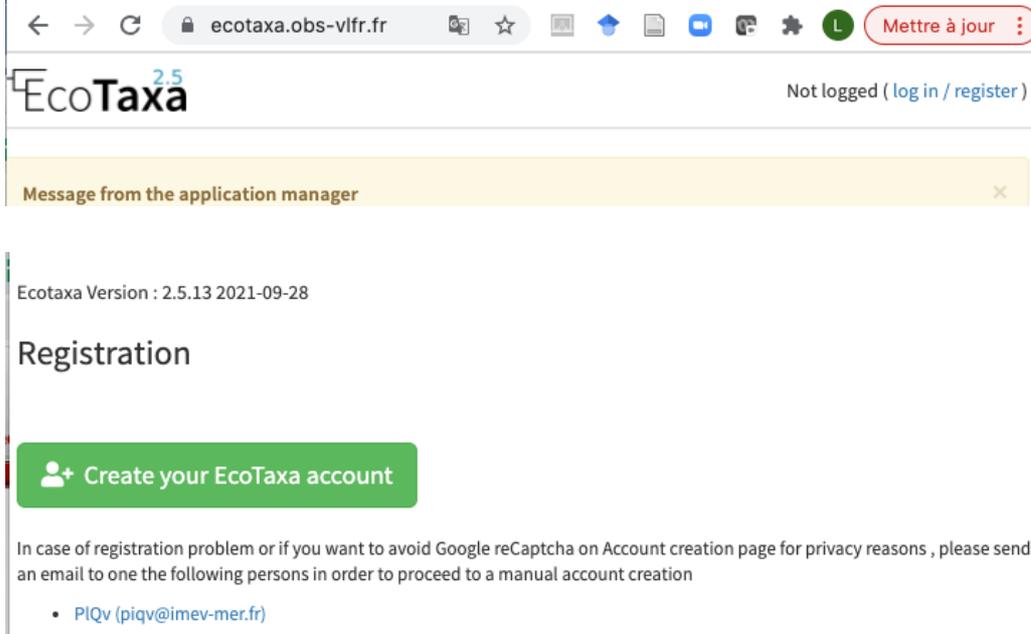
- 8
 1. Drain the sample out of the syringe
 2. Disconnect the syringe and clean it with tap water (or even distilled water)
 3. Pump (**at high speed!**) the full content of the fluidic system to remove any liquid
 4. Reconnect the syringe
 5. fill it with tap water (or distilled water)
 6. pump (**at high speed!**) while regularly pinch the tubing to unstuck any plankton in the system (see [🔄 go to step #5.7](#))
 7. Drain again the syringe (**repeat steps 7.2-7.7 at least 2 times** more until no plankton is visible on the camera)
 8. Finally drain the system

Upload your images on EcoTaxa



At First connection:

Create an account on EcoTaxa (<https://ecotaxa.obs-vlfr.fr/>) by clicking on the top right "log in/register"



← → ↻ ecotaxa.obs-vlfr.fr        Mettre à jour ⋮

EcoTaxa^{2.5} Not logged ([log in](#) / [register](#))

Message from the application manager ×

Ecotaxa Version : 2.5.13 2021-09-28

Registration

 [Create your EcoTaxa account](#)

In case of registration problem or if you want to avoid Google reCaptcha on Account creation page for privacy reasons , please send an email to one the following persons in order to proceed to a manual account creation

- [PIQv \(piqv@imev-mer.fr\)](mailto:PIQv@imev-mer.fr)

Put your real name and a valid mail to contact you

Create an EcoTaxa account

First name * **Last name ***

Email address *

Password * **Password confirmation ***

Warning: This server does not use encrypted communication (https) for performance reasons. This means that your password can be collected by a third party spying on the connection. Be careful which password you use here.

Organisation *

Country *

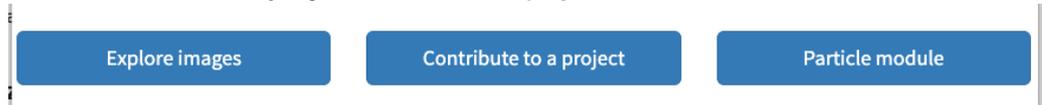
Planned usage of EcoTaxa

I agree with the following usage conditions: *

- I will make a reasonable use of the resources of this system.
- I accept that my annotation activity and statistics are tracked and shared with the members of the projects I participate in and the EcoTaxa team, for the application to function.

* Required fields

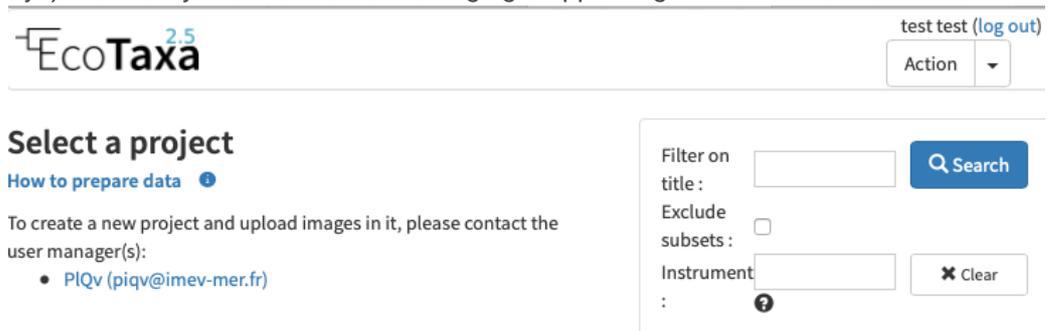
9.1 Once logged you can consult the project on which you are registered (e.g. your own projects + the ones you have been invited by the different data owners) by clicking onto **"contribute to a project"** on the main page



9.2 **Needs to be done only once:** basic rights don't include the "create project". As a protection against bots, To create a new project and upload images in it, please contact the user manager(s):

- [PIQv \(piqv@imev-mer.fr\)](mailto:PIQv@imev-mer.fr)

The rights to create projects will be activated (by a human, please be patient few days) soon and you will see the following right appearing



The screenshot shows the EcoTaxa 2.5 interface. At the top right, there is a user profile for 'test test (log out)' with an 'Action' dropdown menu. Below this is a 'Select a project' section with a link 'How to prepare data' and a list of user managers, including 'PIQv (piqv@imev-mer.fr)'. To the right of this section is a search filter box with fields for 'Filter on title:', 'Exclude subsets:' (with a checkbox), and 'Instrument:'. There are 'Search' and 'Clear' buttons in the filter box.

without the right to create projects

Select a project

[How to prepare data](#) ⓘ

Create a new project

Filter on

Exclude subsets :

Instrument

: ⓘ

...

with the right to create projects

- 9.3 You can now create your own project on which you will be able to import, visualise and classify images

Create a new project

Project Title :

Create the project

Cancel

- 9.4 upload the ecotaxa archives (see step 6-7) on the EcoTaxa ftp using Filezilla (see [go to step #7](#)) create a connection by using the following informations:
Select File > Site Manager...

Create a New Site called : Ecotaxa_VLFR

In General tag :

Host : plankton.obs-vlfr.fr

Protocol : FTP – File Transfer Protocol

Encryption : Only use plain FTP (insecure)

Logon Type : Normal

User : ftp_plankton

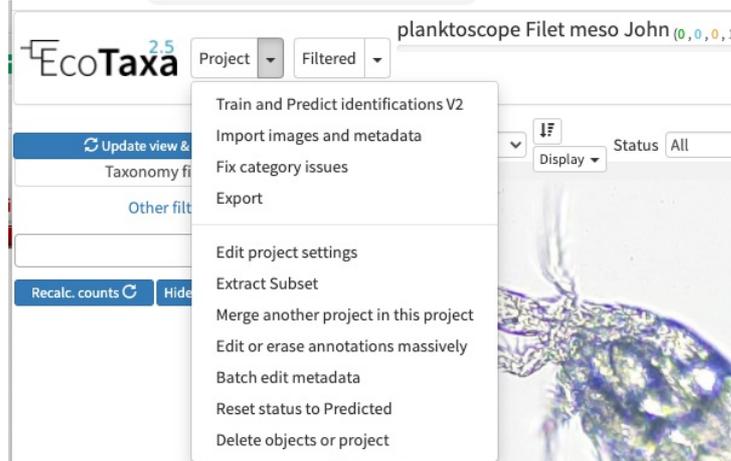
Password : Pl@nkt0n4Ecotaxa

this done you could use FileZilla to load the Zip files downloaded from the Planktoscope onto the EcoTaxa ftp server (e.g. /Ecotaxa_Data_to_import/PLANKTONSCOPE)

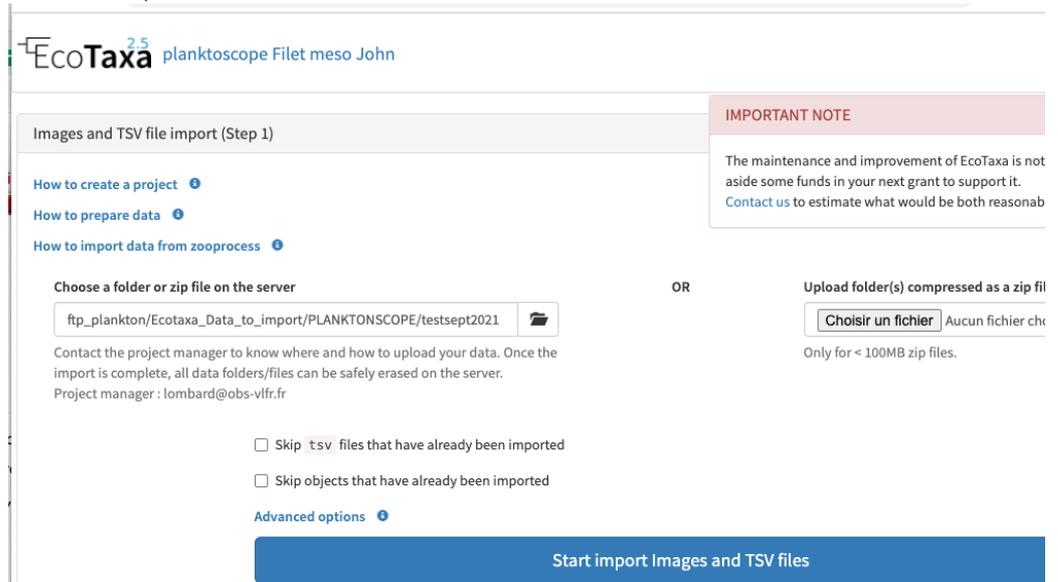


- Please eventually create your own folder to "try" to keep it clean and tied
- Please think to regularly remove those temporary files from the ftp, at this point they are not secured at all and everybody can access them (and disk space is not free)

9.5 In your project/ on your project options button, select import images and metadata



9.6 locate your file on the ecotaxa ftp folders and import it (only works one zip file at a time for now)



How to use efficiently ecotaxa

10 Configure your project efficiently: in Project/project settings

- Select a data sharing license (we recommend one of the CC-BY one or CC-0 if you want data to have a future use for science)
- Define if the project is visible for visitors (only "validated" images will be visible)
- Add a preset list of taxa for manual sorting (could be copied from any other project, or taxa added manually): those will be present in the taxonomic filter (see 10.1)
- Add useful sorting variables : in "Fields available for sorting": add at least those parameters that are pretty useful and will be added to the Quickfilters (see 10.1)

area=area

meanhue=meanhue

meansaturation=meansaturation

meanvalue=meanvalue

Edit project # 4891 - [Edit privileges only](#)

Project Title

Data sharing license
 CC-0: all registered EcoTaxa users are free to download, redistribute, modify, and build upon the data, with no conditions. Other databases can index the data. The data falls into the worldwide public domain. This is the license preferred by OBIS and GBIF.
 CC-BY: all registered EcoTaxa users are free to download, redistribute, modify, and build upon the data, as long as they cite the dataset and its authors. Other databases can index the data.
 CC-BY-NC: all registered EcoTaxa users are free to download, redistribute, modify, and build upon the data, as long as they cite the dataset and its authors, and do not use it for commercial purposes (primarily intended for or directed toward commercial advantage or monetary compensation"). Other databases can index the data.
 Copyright: only contributors to this project have rights on this data. This prevents its distribution in any kind of database.
 Not chosen

Visible for all visitors (only validated objects)

Status

Project Description

Definition of preset for manual sorting

Fields available for sorting & Display In the manual classification page

area=area
 meanhue=meanhue
 meansaturation=meansaturation
 meanvalue=meanvalue

- Define a person of contact (mandatory)
- define what pre-trained Deep Learning features to use on your project (we recommend to use «flowcam » unless we start to have some trained on planktoscope image

SCN Network

SCN Network used for prediction, if you change it, existing features computed with this network will be erased. New features will be recomputed during the next automatic classification.

- And invite people to manage the project with you (will have the same rights than you) helps you to annotate images (won't have options below "export" in the project see [go to step #9.4](#)) or just viewing the project (both validated and non validated)

Privileges

Name	Privilege	Contact	Delete
Fabien Lombard x v	Manage v	<input type="radio"/>	<input type="checkbox"/>
Adam Larson x v	Manage v	<input checked="" type="radio"/>	<input type="checkbox"/>
Manu Prakash x v	Manage v	<input type="radio"/>	<input type="checkbox"/>
Thibaut Pollina x v	Annotate v	<input type="radio"/>	<input type="checkbox"/>
test test x v	View v	<input type="radio"/>	<input type="checkbox"/>
New privilege:			
search user...	View v		

10.1 Use filters wisely

there is three layers of filters in EcoTaxa: the quick access filters (top bar)

Select all area Display Status All 100 % 1 Selected

The taxonomic filter tab (allows to filter by taxonomic groups) and the other filter tabs

EcoTaxa 2.5 Project Filter

Update view & apply filter

Taxonomy filter ⓘ Other filters

Asterionellopsis	0	11
Chaetoceros socialis < Chaetoceros	0	1
▼ Coscinodiscophycidae	8	47
Bacteriastrum < Chaetocerotales	0	36
Chaetoceros < Chaetocerotales	0	41
▼ Dinophyceae	0	741
Ceratum sp.	0	644
Neoceratium 01	0	601
Neoceratium furca < Neoceratium	0	108
Neoceratium pentagonum	0	91
Noctiluca < Noctilucaeae	0	12
Oxytoxum	16	245
Pyrocystaceae	0	2
Pyrophacus	0	4
Ditylum	0	78
Eucampia	0	2

taxonomic filters

EcoTaxa 2.5 Project Filter

Update view & apply filter

Taxonomy filter ⓘ Other filters

Share page Clear all filters

Sample Advanced Clear

Sample

Depth ⓘ Clear

Min [m] Max [m]

Location Clear

West North East
South

Open map

Date Clear

Begin

End

Month

Time invert Clear

Begin End

Day time

Instrument ⓘ Clear

Instrument

Annotator / Free filters Advanced Clear

search user...

Validation date begin

Validation date end

Num. field >= <=

Text field contains

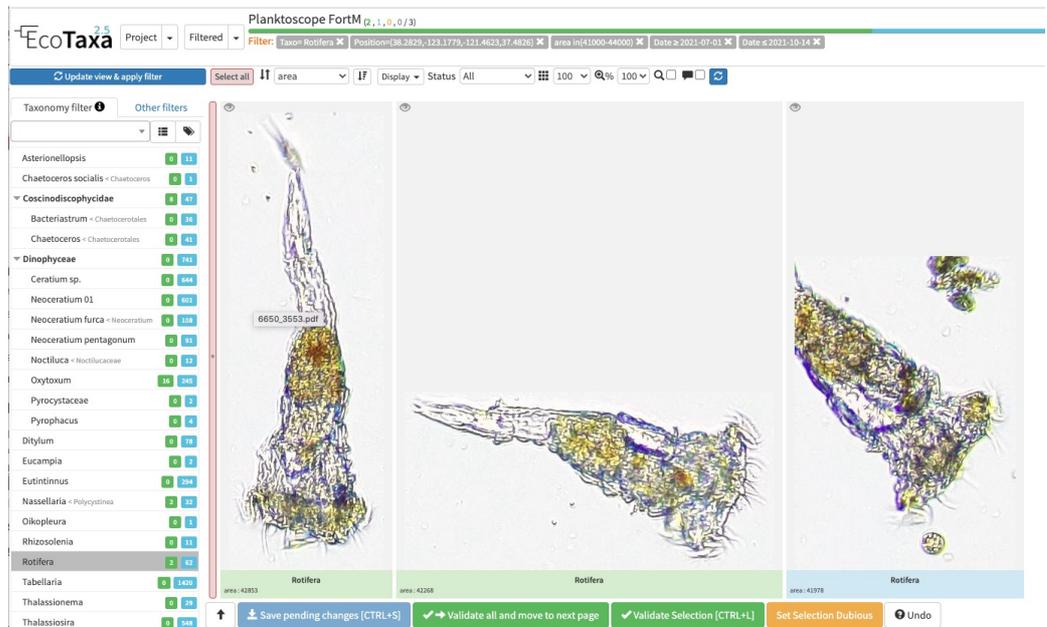
Update view & apply filter

Save filters

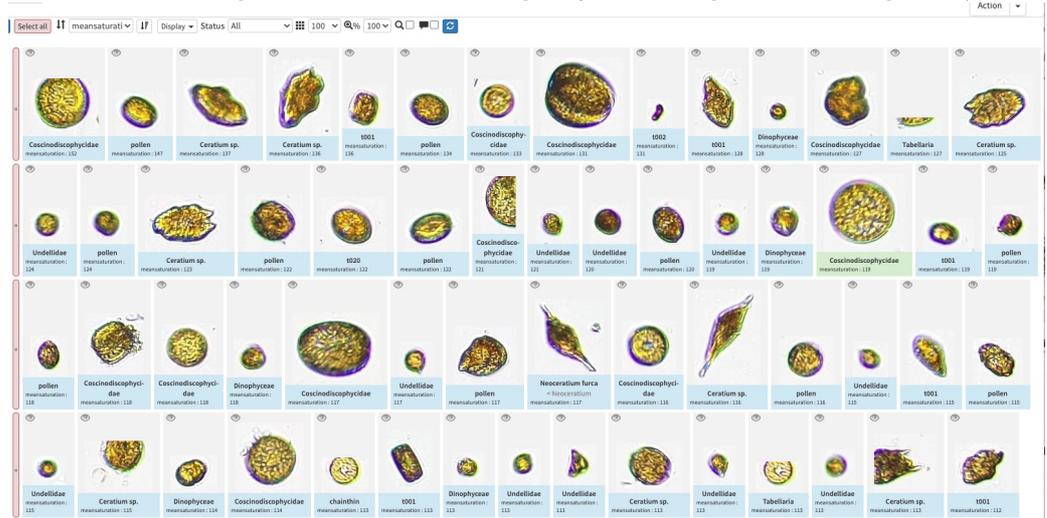
Apply saved filters and update view

other filters

Filters are additive, so you can addition filers on geography, date, who validated them, taxonomic group and every numeric fields/ text fields entered in ecotaxa to search for specific things (and you can get rid of them easily too, see grey fields on to of the next image)

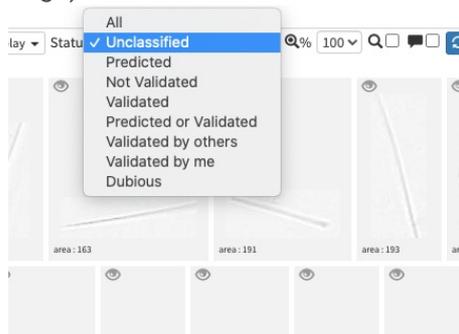


quickfilters are pretty useful since you can sort object by specific values (eg. mean saturation bellow) to quickly observe object that have here lot of chlorophyll (ps. you can revert the sorting order of those filter to get by ascending or descending order)



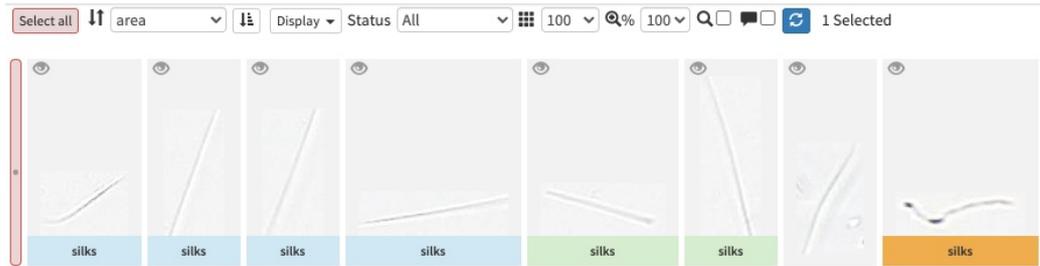
10.2 The different validation "states" in ecotaxa and how to validate

image arrives in EcoTaxa with the status "unclassified" (grey surrounding of the image)



However they could be also set as "predicted" (blue surrounding; classified automatically by taking as example one pre-existing project), "validated" (green

surrounding; checked and annotated by a human), or dubious (orange surrounding; checked and annotated as dubious by a human)

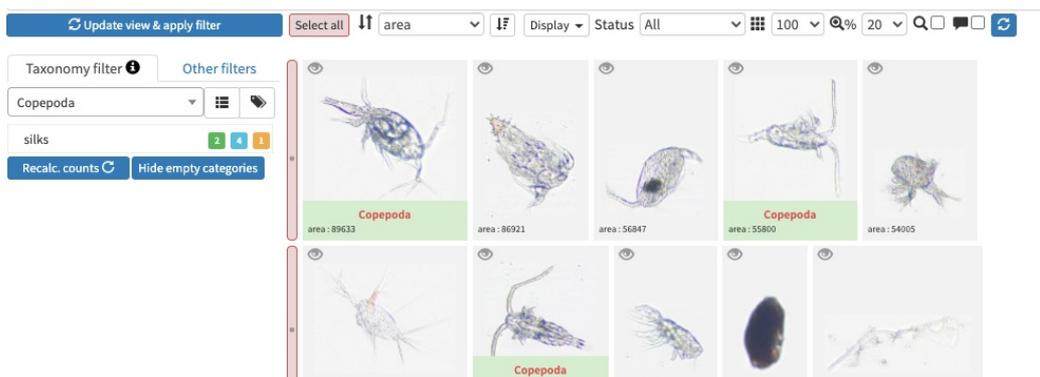


-validating consist in selecting one or several picture and attributing them a taxonomic or morphological identity by either displacing them in the list of taxa present in the « taxonomic filter » tab (in which you can force some categories to be present by using the « preset » in the project settings... or just by typing the name using the keyboard (which should use right away the research on top of the »taxonomic filter ». Whatever happens you need to save (ctrl.S or save button at the bottom of the page) before your action gets finally implemented.

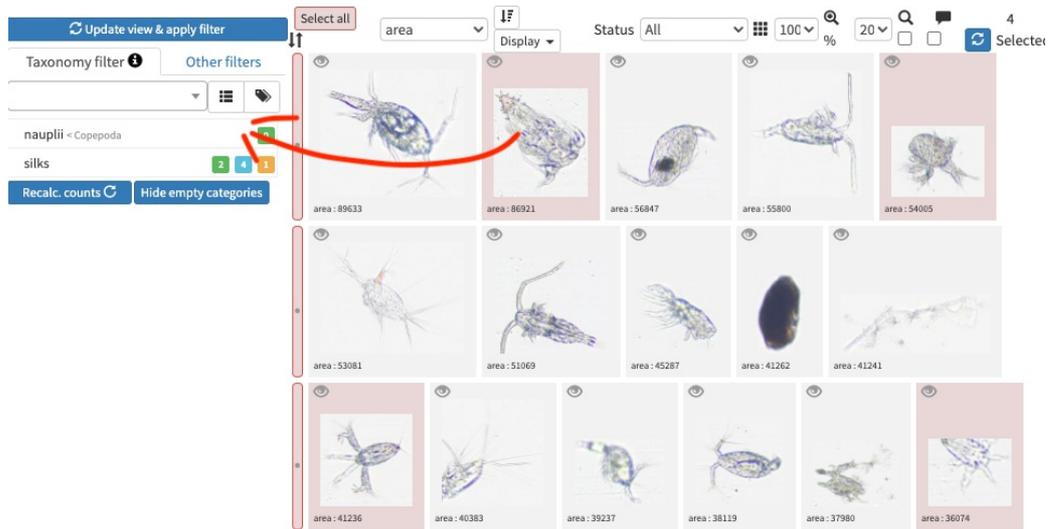
photo here to illustrate



typing "copepo" brings several results



once validated the name appears in red below the images



sliding into existing categories does also works



dont forget to save your validations

-validation could be tedious and requires large taxonomic expertise, however there is plenty of tools to help you! Filters are one of those tools, but the more interesting one is to use previous project to « predict » some taxonomic identity on your new images, in best cases you will face thousands of rightly predicted images and will be able to validate thousands per minutes!

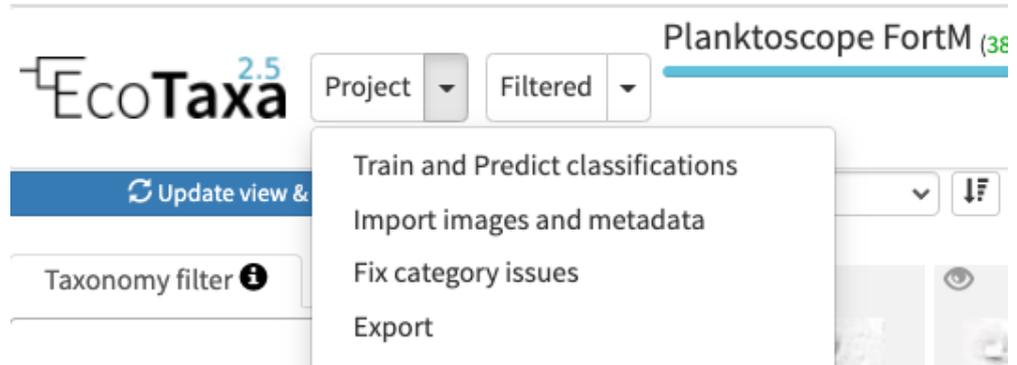


example of (well) predicted objects which would be easily validated

10.3 Do not hesitate to "predict" your project right away (even with a project/instrument that have nothing to do)

-how to do prediction (and helps you to boost your validation ability)

In the project (or "Filtered", in this case only the filtered vignettes would be used), select "train and Predict identification"



- You can select any of the pre-existing project (including your own project) as a template for image recognition. By experience, using another project is only a first-aid, but won't replace prediction on images that you acquired with the same instrument/ same location / same plankton communities
- Note that currently, only few "sorted" planktoscope projects do exists (especially acquired with the same segmentation procedure than here), we therefore strongly encourage you after a first trial of prediction to quickly validate to re-predict on your own project.

■ what could be used for first prediction :

#4605 - Planktoscope NOAA WCOA21 rita-net (coastal US West coast from Vancouver to San Diego; Processed with current segmenter; Partly validated; **Contains lots of artefacts due to lens mis-alignment** see 5.1)

- #4356 - Planktoscope Tara Microbiomes P2/miniHSN : Lorient > Punta Arenas V3 (Trans-atlantic transect from France to Ushuaia; Processed with other segmenter (**works only with adding Deep Learning features into play**); Fully validated)

PREDICTION: Choice of Learning Set data source

Next: Choose objects in selected projects

A Learning Set AKA Training Data Set is built using validated data from one or several projects. Inside these projects, you will be able, in next pages, to specify:

- Which categories you're interested in predicting.
- How many objects you want to use as reference for the prediction.
- For these objects, which features are relevant to the prediction.

This Learning set will then be used for running the prediction task.

Filter on title: # Matching features \geq 10 Instrument:

Project deep features model: flowcam. Model is **usable**

# - Title	# Validated	# Matching features	Deep features model
<input checked="" type="checkbox"/> #4655 - Planktoscope FortM	38	38	flowcam
<input checked="" type="checkbox"/> #4605 - Planktoscope NOAA WCOA21 rita-net	16373	38	flowcam

- Push the button "select project bellow then click me. You then have the possibility to select what types of object to consider. It is recommended to try to avoid selecting too much object to partly correct the usual strong imbalance between categories (here as an example limited to 100 example per groups) (If you use project #4605 as an example, please remove artefacts)

PREDICTION: Choice of Learning Set categories and size

Next: Choose features in selected objects

From data source, which is : #4655 - Planktoscope FortM, #4605 - Planktoscope NOAA WCOA21 rita-net, only objects validated in below chosen categories will be present in the Learning Set being built. Optionally, each category can appear as another category, generally a parent one, to the machine learning algorithm.

- The experience shows that it is often more efficient to predict into a limited number of categories and then validate in detail using more categories.

Learn from max objects per category. Total is currently 2877 objects.

Make categories appear like in:

(id)	Source (validated) category	# source	% source	# learning set	Appear as category
(85008)	<input type="checkbox"/> artefact	13036	79.4 %		<input type="text"/>
(92010)	<input checked="" type="checkbox"/> silks	998	6.1 %	500	<input type="text"/>
(85079)	<input checked="" type="checkbox"/> multiple < other	335	2.0 %	335	<input type="text"/>
(85352)	<input checked="" type="checkbox"/> chainthin	198	1.2 %	198	<input type="text"/>
(92767)	<input checked="" type="checkbox"/> nauplii < Copepoda	174	1.1 %	174	<input type="text"/>
(89190)	<input checked="" type="checkbox"/> Ceratium sp.	167	1.0 %	167	<input type="text"/>
(28249)	<input checked="" type="checkbox"/> Ditylum	118	0.7 %	118	<input type="text"/>
(28117)	<input checked="" type="checkbox"/> Tabellaria	108	0.7 %	108	<input type="text"/>

- click on "continues to the classifier option screen ". Activate the pre-trained Deep

Learning features (if not available see step 10). Inactivate variables that are not relevant for prediction and relates to position of the vignette into the initial images (bx, by, depth min/max, label, local centroid col/row, x, y)

PREDICTION: Choice of features and settings

Start prediction task

You have chosen 2877 reference objects to build the Learning Set. In this last step, you can choose which features to associate with each of these objects, and start a prediction task using the Learning Set.

- Prediction will be better if you exclude features which are not related to the classification, e.g. coordinates in the raw image.
- Features with a single, constant value, or too many missing values, are useless for prediction and are automatically excluded. Some of them are listed here as a reminder.
- Missing values will be replaced by the median value for this feature from the reference objects.
- Prediction settings are recorded in EcoTaxa for the next prediction.

Add deep features ✔

%area

angle

area

area_exc

bounding_box_area

bx

by

circ.

circex

convex_area

depth_max

depth_min

eccentricity

elongation

equivalent_diameter

euler_number

extent

height

label

local_centroid_col

local_centroid_row

major

meanhue

meansaturation

meanvalue

minor

perim.

perimareexc

perimmajor

solidity

stdhue

stdsaturation

stdvalue

width

x

y

Once done images are now "predicted" (blue) but still awaits validation. While classifying the different objects, the classifier also gave a classification "score" which determine if the label is attributed with high or low confidence. Using this score as a quick-filter is usually a good idea to be able to validate quickly well recognised images (and quickly start new predictions)

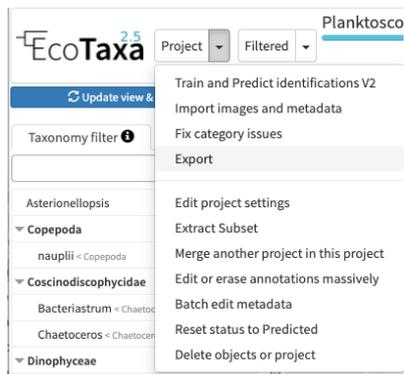
The screenshot shows the EcoTaxa 2.5 interface. At the top, it says 'Planktoscope FortM (0, 644, 0, 0 / 644)'. The 'Filter' is set to 'Taxo=Ceratum sp.'. The left sidebar shows a taxonomy filter with 'Ceratum sp.' selected, showing 644 objects. The main area displays a grid of 36 predicted images, each with a score. The scores are: 0.91, 0.89, 0.83, 0.83, 0.82, 0.81, 0.80, 0.80, 0.79, 0.78, 0.76, 0.76, 0.76, 0.76, 0.75, 0.75, 0.74, 0.74, 0.73, 0.70, 0.70, 0.70, 0.70, 0.70, 0.69, 0.69, 0.68, 0.68, 0.68, 0.68, 0.67, 0.66, 0.66, 0.66, 0.66, 0.65, 0.65, 0.65.

example of images sorted by score of prediction

Doing repeated predictions on your own samples is better than doing some global one on random example project

Quickly validate objects to start to predict on your own plankton composition: the classifier is quite efficient and starts to give reasonable results starting from 30-50 images as example. Ecotaxa is then optimised to operate regular prediction rounds which could be heavily guided by the human (e.g. by doing prediction only on selections, stopping to predict some organisms etc).

-once fully validated, export your results (lots of different solutions does exist, the more easy to understand being the summary export with count per sample)



EcoTaxa 2.5 Planktoscope FortM

Data Export

Export format	Options
<input type="radio"/> General export (configurable export for general purposes)	<input checked="" type="checkbox"/> Object Data (median,mean, x, y, ...) <input checked="" type="checkbox"/> Process Data (software,version, ...) <input checked="" type="checkbox"/> Acquisition Data (Resolution, ...) <input checked="" type="checkbox"/> Sample Data (lat,long, date, ...) <input type="checkbox"/> Historical Data <input type="checkbox"/> Comments <input type="checkbox"/> Use coma as decimal separator <input type="checkbox"/> Format dates and times using - and : <input type="checkbox"/> Internal Ids (including taxonomic source Id) Split in multiple files by: NOT Active
<input type="radio"/> Backup export (ready to re-import datasets, possibly including images)	<input type="checkbox"/> Export all image files
<input type="radio"/> D.O.I. export (ready to publish datasets)	Export image files : NO Images To archive a dataset in a public repository for future scientific exploitation (and possibly attach a DOI to it), use this option. It will export all classifications in an easily readable, non-ambiguous format as well as all the metadata associated with each object. If you choose to export images, they will be sorted taxonomically in subfolders. Beware, however, that many online repositories may not accept to host the images because of their size.
<input checked="" type="radio"/> Summary	Count per category and Sample

Maintenance of your planktoscope

11 clean tubing and flowcell from inside

imaging plankton will lead to have a lot of organic material and seawater in the fluidic system. Some may clog or accumulates in some parts of the fluidic system.

1. Don't let it dry and try to get rid of it as soon as possible (if its occurs during sample acquisition, even abort this latter, take care of the clog, maybe dilute the sample [↻ go to step #5.3](#) and restart acquisition while noting that the sample get diluted in the metadata [↻ go to step #5.4](#))
2. Pump tap or distilled water with high pumping rates helps to unclog the system. make sure no plankton organisms remain in the fluidic system and especially on the internal walls of the flowcell. If it is the case don't hesitate to pinch (during 1-2 second) and release the tubing between the flowcell and the pump while pumping to creates sudden variation of pressure (e.g. [↻ go to step #5.7](#))
3. Over time, wet conditions and organic mater may create favorable condition for the growth of a bacterial film. The flowcell and tubing will looks dirty from the inside. You can avoid this by pumping diluted bleach sometimes, let it acts for 1-2 hours and carefully rinsing the whole system [↻ go to step #8](#)
4. Water, bacteria, and bleach together may favour the apparition of a calcium carbonate film inside the tubing and flowcell. It may either appears as dispersed cristals attached inside the flowcell or a white coating inside the tubing. To remove and clean this pumps some acidic solution (vinegar, citrus juice or other kind of other acids), let it rest for few hours and rinse the system

↻ go to step #8

clean flowcell outside:

The flowcell is an optical critical component, keeping it clean is an absolute necessity. Don't touch it with fingers or other kind of dirty material. If dirty:

1. if dry dusts only are present, gently blow the flowcell (ideally with dry gas dispenser at a large distance - dry gas dispenser are also creating thermal chocs if used from too close, test it on other material before)
2. if dirt in not only dry dusts it could be cleaned with optical paper and ethanol. **DO NOT USE CLASSICAL WIPING PAPER** which are usually enriched in silica fibers for solidity ... and may create scratches on the flowcell. (disposable nose tissue are better alternative if optical paper is not available)

clean optical lenses

as for the flowcell, optical lenses are critical elements of your planktoscope and should be kept as clean as possible. It starts by never touching them with fingers (cleaning those would requires lot of patience, efforts and may even lead to unexpected disappointments)

1. dry dust: dry gas (with even more caution than previously)
2. others: only used optical paper

clean the camera sensor

... critical part if any, NEVER touch it, only use dry gas

regularly calibrate the pump ↻ go to step #2

To get further

12

external links

13 Planktoscope website
<https://www.planktoscope.org/>

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

Planktoscope complete assembly guide and complete documentations
<https://planktoscope.readthedocs.io/en/latest/>

Planktoscope Slack channel (to exchange ideas/protocols/solutions)
<https://forms.gle/qvh5jwuMvmyBKMQC7>

Plankton Planet website
<https://planktonplanet.org/>

EcoTaxa tutorials:
<https://sites.google.com/view/piqv/ecotaxa?authuser=0>
<https://www.youtube.com/watch?v=PSO6ZS765tk>
<https://www.youtube.com/watch?v=RaWUqIoKk0E>