



Planktoscope protocol for plankton imaging V.1

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DISCLAIMER

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

ABSTRACT

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

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

ATTACHMENTS

Pollina et al 2020

2020.04.23.056978v1.full.
pdf

IMAGE ATTRIBUTION

Fabien Lombard, Thibaut Pollina

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Protocol status: In

development

We are still developing and
optimizing this protocol**Created:** Oct 06, 2021**Last Modified:** Mar 04,
2022**PROTOCOL integer ID:**
53848**Keywords:** Planktoscope,
plankton**GUIDELINES**

Planktoscope is an optical instrument. As its optical elements (camera, lenses, flowcell) are highly sensible 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).

A complete manual of assembly and software can be found at

<https://planktoscope.readthedocs.io/en/latest/>

MATERIALS

Plankton net

200µm sieve

Squeezing bottle

micrometer slide (or millimetric ruler)

Optical paper

Dry gas dispenser

SAFETY WARNINGS

- Planktoscope is an electronic device, powered with electricity. It is therefore sensible to water.
- Place it in an environment where water cannot 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).

BEFORE START INSTRUCTIONS

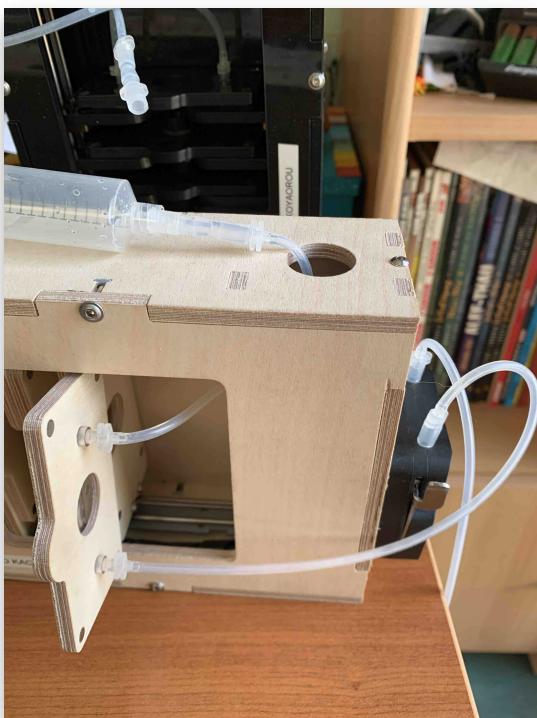
- Test the protocol before acquisition of your first sample
- Calibrate your instruments to ensure coherent measures
- Create an Ecotaxa account and request the right to create project way before
- Collect a plankton sample using a net

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

Safety information

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

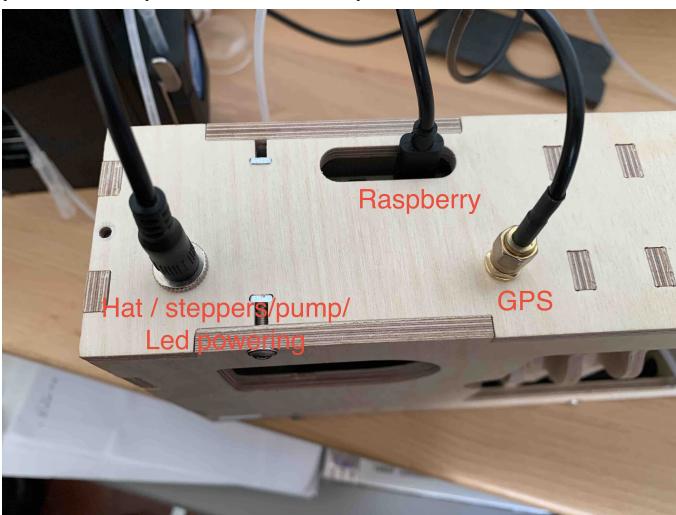
If these steps are not followed properly, plankton will have the tendency to sediment and will progressively form clogs in the fluidic system which may severely lead to undersampling of plankton

- Choose tubing which is the smallest as possible (eg. if flowcell is 200µm wide, tubing with internal dimensions >1mm is not needed and will create 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 has a switch, put it on.



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

Note

it is possible to connect by ethernet instead if you 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>

Safety information

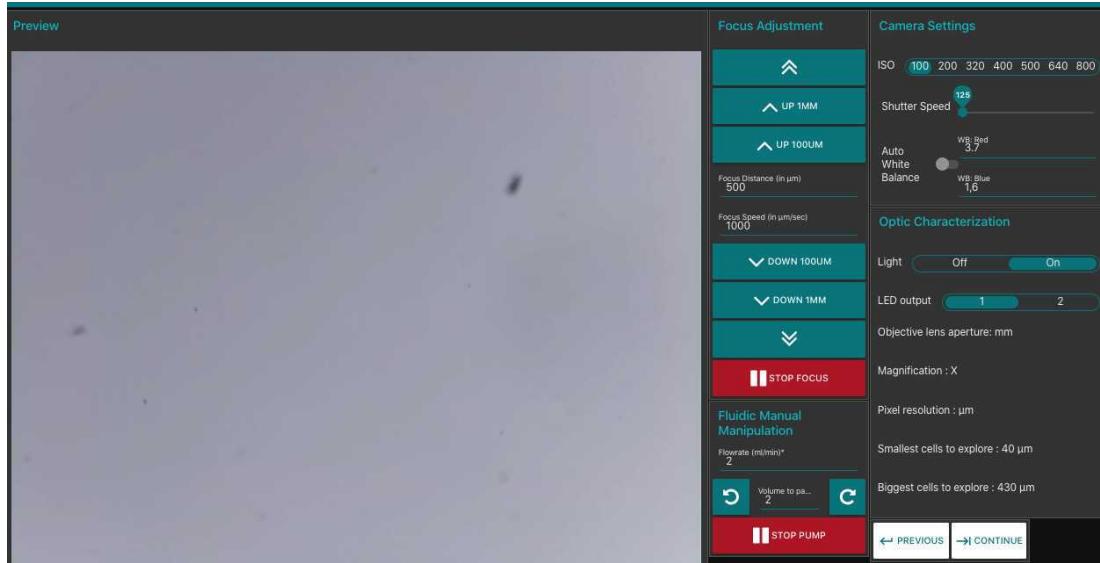
For software versions previous to 2.3

- For windows users, you may need to download the "bonjour" printing system to communicate with the planktoscope: see here : <https://support.apple.com/kb/dl999>
- For mac users, this shouldn't be a problem
- for linux, we haven't tested it yet and may not have a solution

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

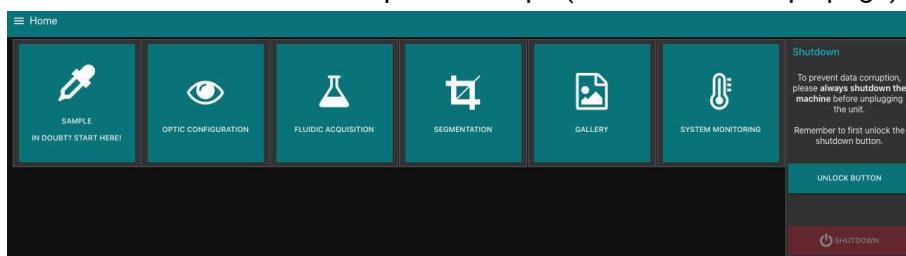
- 1.4** Check that your planktoscope is properly operating: on the optic configuration page:

- turn the light on/off (you should see the light and the camera overview turning white)
- check that adjusting the focus makes the focus motor 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 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 lead to incorrect results)
- 1.6** Locate how to shut down the planktoscope (main Planktoscope page)



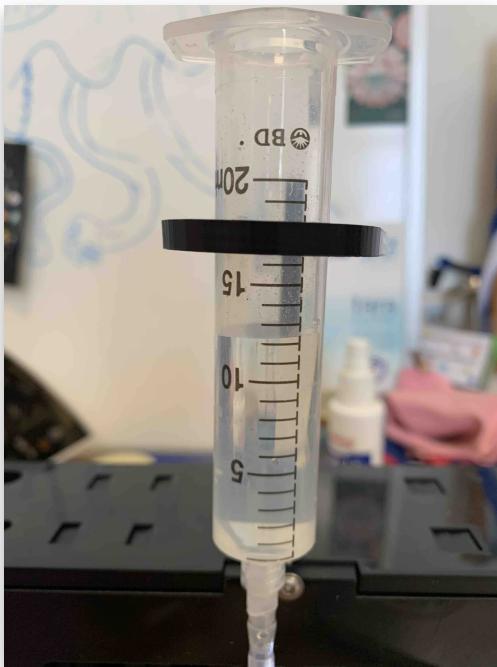
Calibration

2 Pump calibration:

Safety information

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 it is the number of images (therefore the volume imaged) which is important (not the pumped volume)

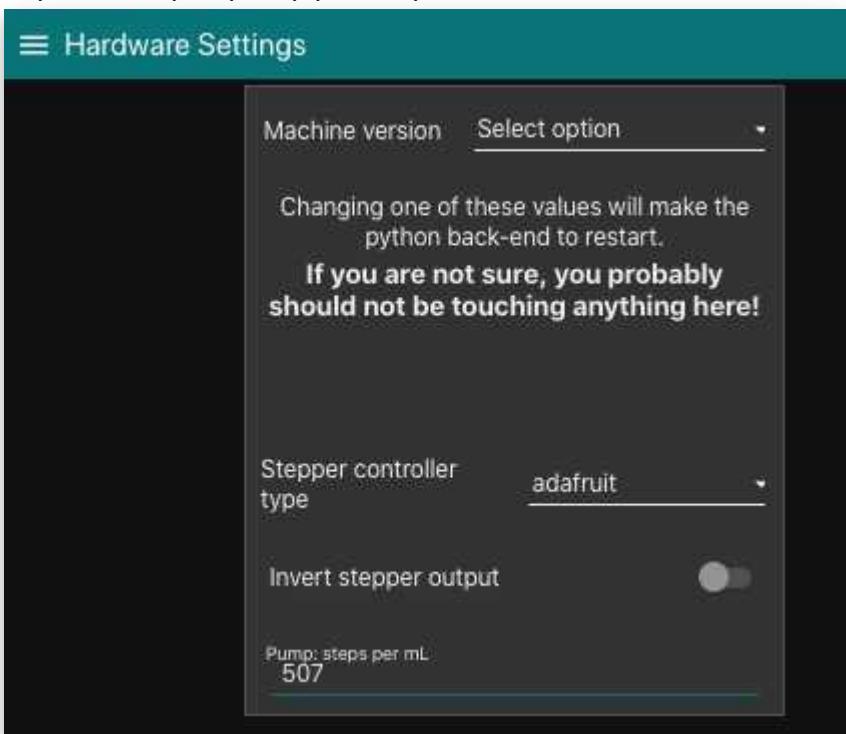
- 2.1**
- prepare a large volume of tap water and put in in the syringe targeting a total volume of e.g. 20ml
 - on the optic configuration tab: tell him that you want to pass 10ml and record the exact volume it finally ends to pass (eg. by looking on the graduation of the syringe) note X= final volume 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 = $10 * \text{old step} / X$
- replace the "pump step per ml" parameter with this value



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

3 Size calibration:

Safety information

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

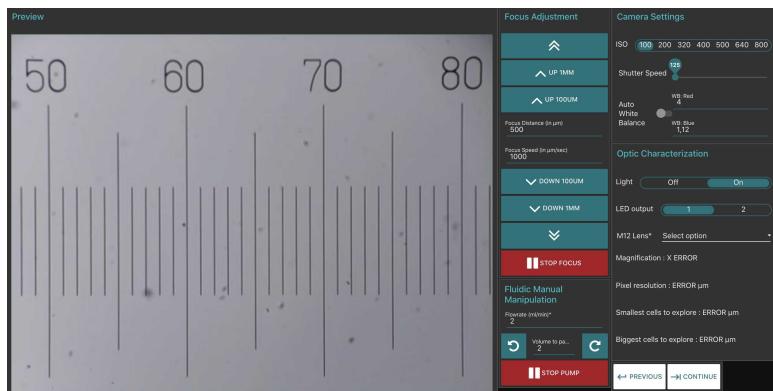
3.2

- 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 the flowcell has been removed)



focusing on the scale

3.3 -take few images (select the test or culture mode)

☰ Sample

Sample Identification

Name of the project*
calibration

Name of the ship
none

Name of the operator*
FL

Station ID*
0

Sampling gear* Plankton net

Sample Location

Latitude (36.574439°N or 36°57.4439'N)
90.0°S

Longitude (110.42100°W or 110°4.2100'W)
0.0°W

Date (YYYY-MM-DD, UTC)
2021-10-08

Time (HH:MM:SS, UTC 24h)*
17:00

VALIDATE **RESET**

PREVIOUS **CONTINUE**

example of metadata entered in "sample" page

☰ Fluidic Acquisition

Preview

Acquisition

Acquisition unique ID*
1

Number of images to acquire
2

Pumped volume (mL)
0.013

Delay to stabilize image (s)
0.2

Total imaged volume
0.01 mL

Flowcell
300 µm capill...

Total pumped volume
0.03 mL

Pump direction

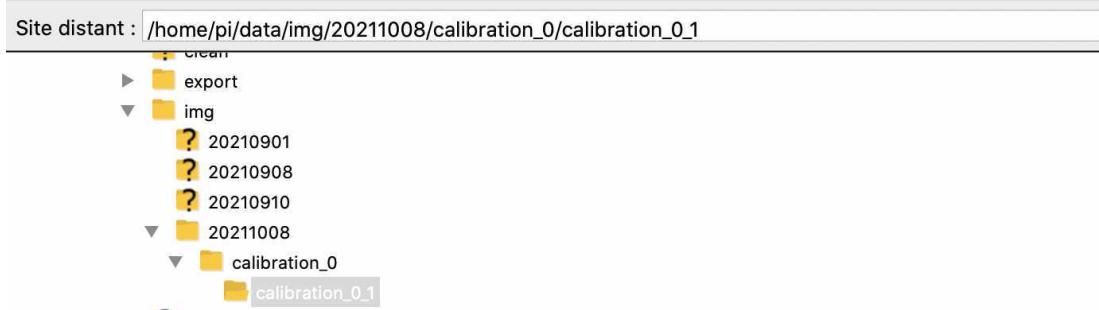
STOP ACQUISITION **START ACQUISITION**

UPDATE CONFIG

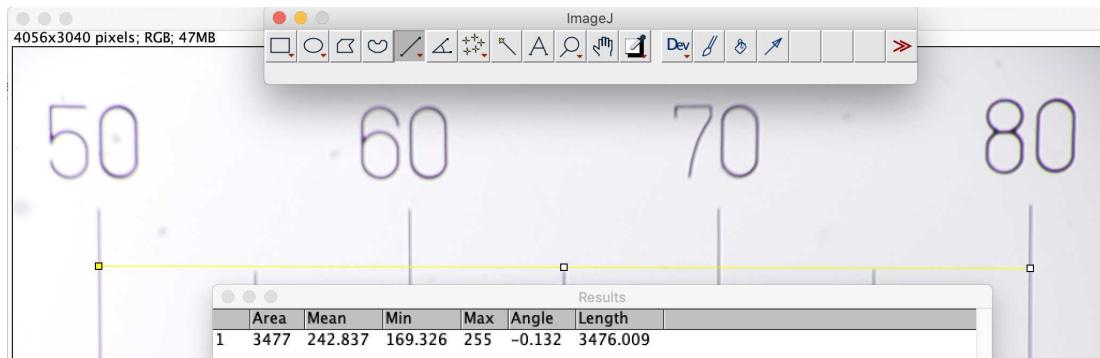
Statistics

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)

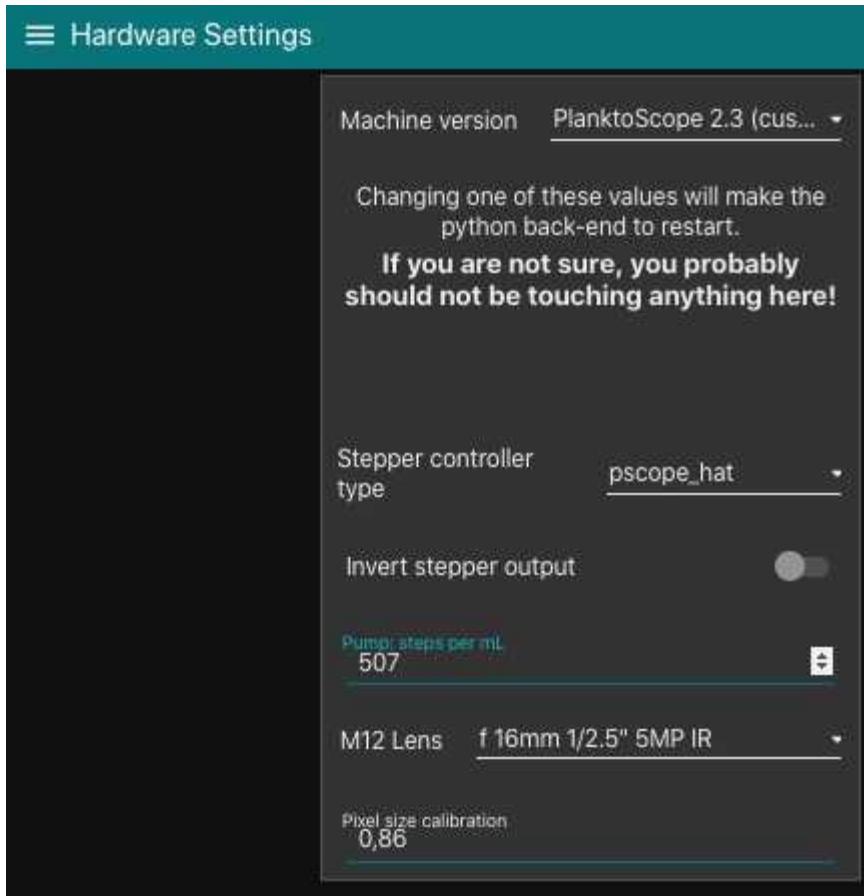


downloading resulting images (using Filezilla)



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 microns are 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

Safety information

Record **Latitude Longitude** (taking photos of the GPS when launching and recovering the net could serve, if UTC time is 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 200 μm sieve

Safety information

larger organisms may clog the flowcell



filtration through a 200 μm mesh



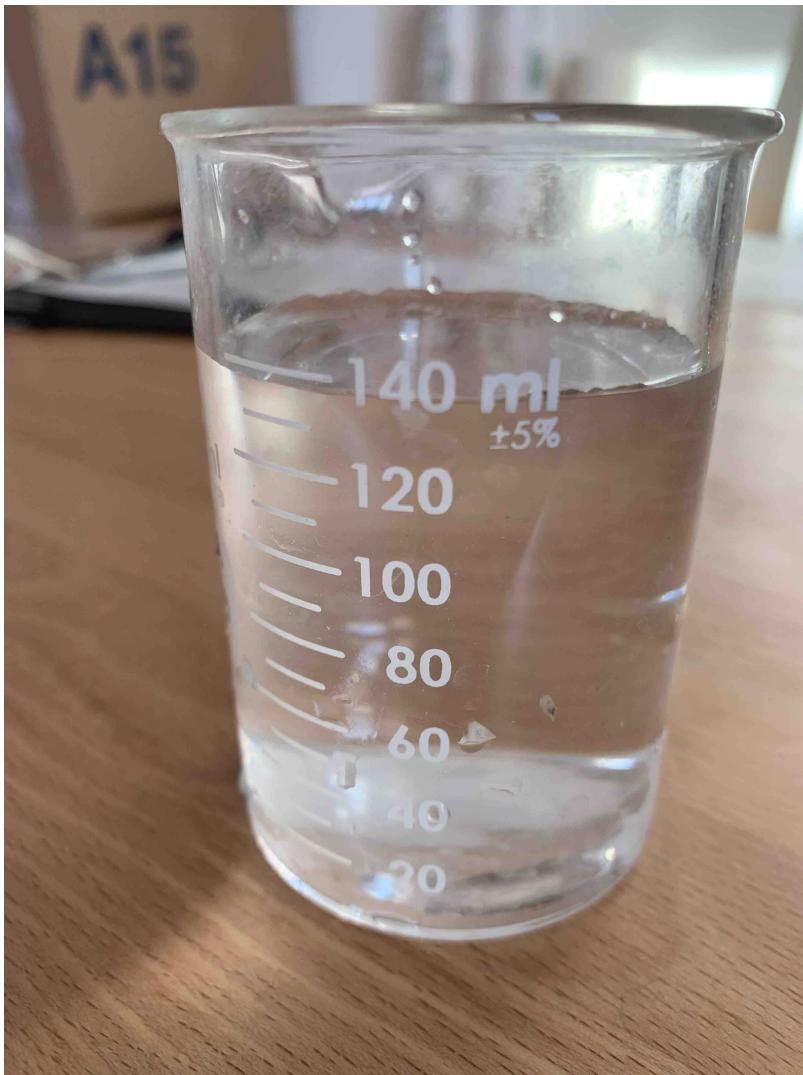
detail of the (home made) filter

4.3 Rinse the sieve using seawater and a squeezing bottle (helps to pass small objects)

4.4 Recover the fluid / measure its volume/ record it on logsheets (will be entered latter as "**concentrated sample volume**")

Safety information

Those are critical informations to get to quantitative sampling



concentrated final volume of plankton

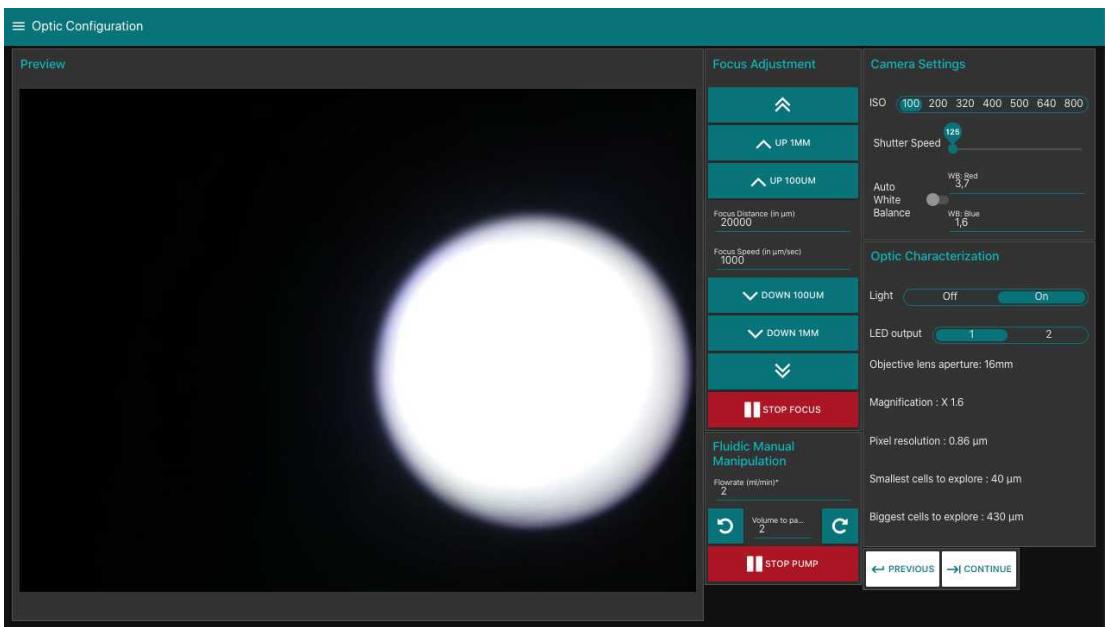
Pass the sample on planktoscope

- 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](#))

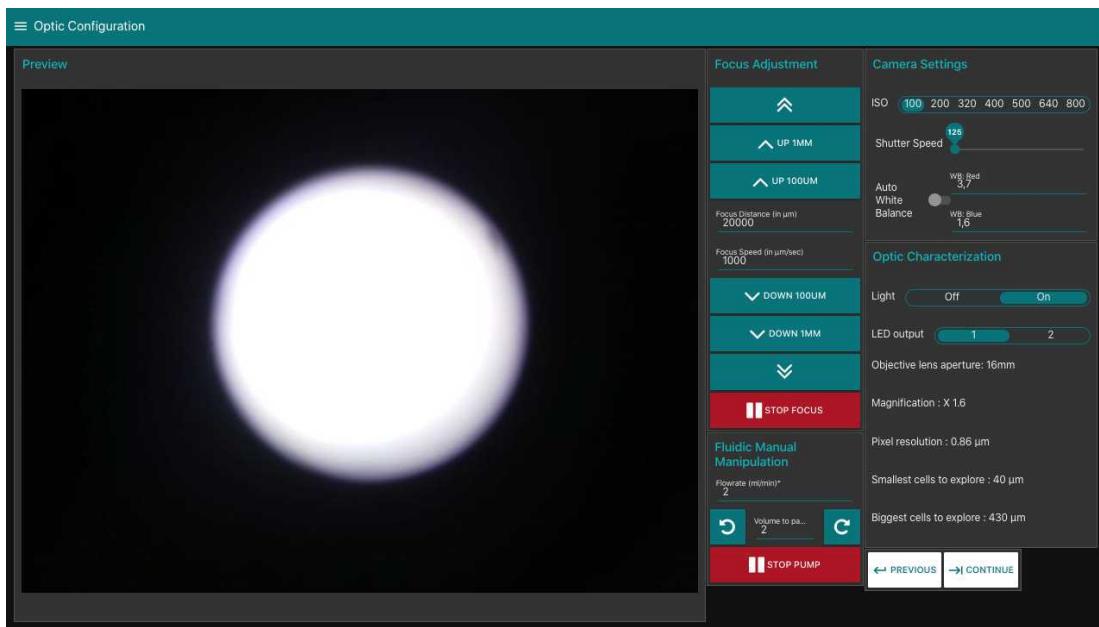
- 5.1 check for lenses alignment : remove the objective lens, start the light and check if the light source is centred.
- if yes place back the objective lens and flowcell
- if no adjust the position of the tube lens to center the light source (magnets allows for 1-2mm adjustments)



objective lens and flowcell removed



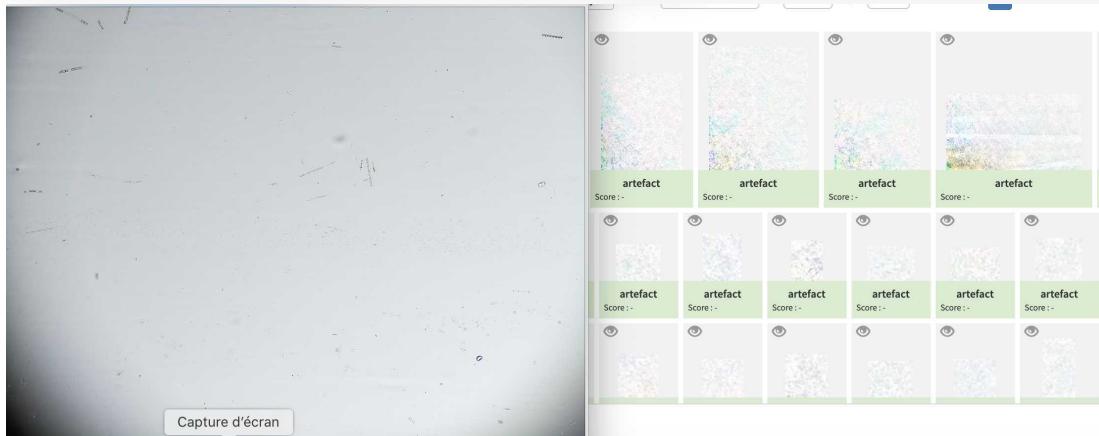
unaligned lens



centered lens

Safety information

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 for this



severely unaligned lens final results 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 by gently inserting 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 finish by typing 25 or 50µm adjustments in the middle box (and pressing external arrows of focus)

Safety information

not agitating your sample will let plankton 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**

Safety information

Having too much object per frame will :

1. increase the probability that objects are touching (making them impossible to count or identify)
2. increased the probability of clogging the fluidic system
3. create 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

-note **how you sampled the plankton** (recording mesh size with "**minimal fraction size**" (will be used afterwards in the segmentation process, objects smaller than this won't 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 a horizontal towed net

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

Dilution factor if a dilution has been done in  go to step #5.3

Sampling gear*	Plankton net	
Minimal fraction size (μm)	<input type="button" value="▼"/> 20 <input type="button" value="▲"/>	Min sampling depth (m) 0
Maximal fraction size (μm)	<input type="button" value="▼"/> 200 <input type="button" value="▲"/>	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 validate 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 images to acquire (to be chosen depending on the desired final object number and the observed concentration on images)

Safety information

pump significantly between two images will help to :

1. avoid plankton sedimentation in the fluidic system
2. avoid imaging two times 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 have 10 objects per image, imaging 100-200 frames would be enough)

Acquisition

Acquisition unique ID*	1	Number of images to acquire	100
Pumped volume (mL)	0.03		
Delay to stabilize image (s)	0.3	Total imaged volume	0.26 mL
Flowcell	200 µm µ-Sli...	Total pumped volume	3.00 mL
Pump direction	Toggle switch	UPDATE CONFIG	
STOP ACQUISITION		START ACQUISITION	

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

Fluidic Manual Manipulation

Flowrate (ml/min)*	5	
	Volume to pa...	
STOP PUMP		

- 5.7** Go to fluidic acquisition and start the acquisition.
 Wait for the acquisition to be done
 Results can be consulted by consulting the gallery

Safety information

If your fluidic system is not optimised to avoid plankton sedimentation, some plankton could accumulate in the fluidic system. This can be checked by pinching 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 this means that plankton have sedimented between the syringe and the flowcell.

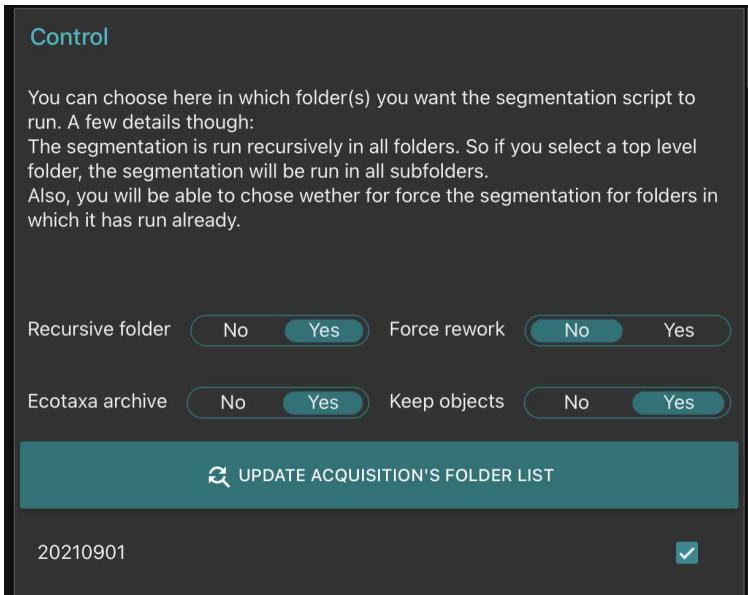


Segment the acquisition

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 keep the final segmented images visible in the planktoscope (that could be accessed by the gallery in the objects folder)



- scroll down and clic on start segmentation
- Wait for the segmenter status to turn to "done"



Download the results

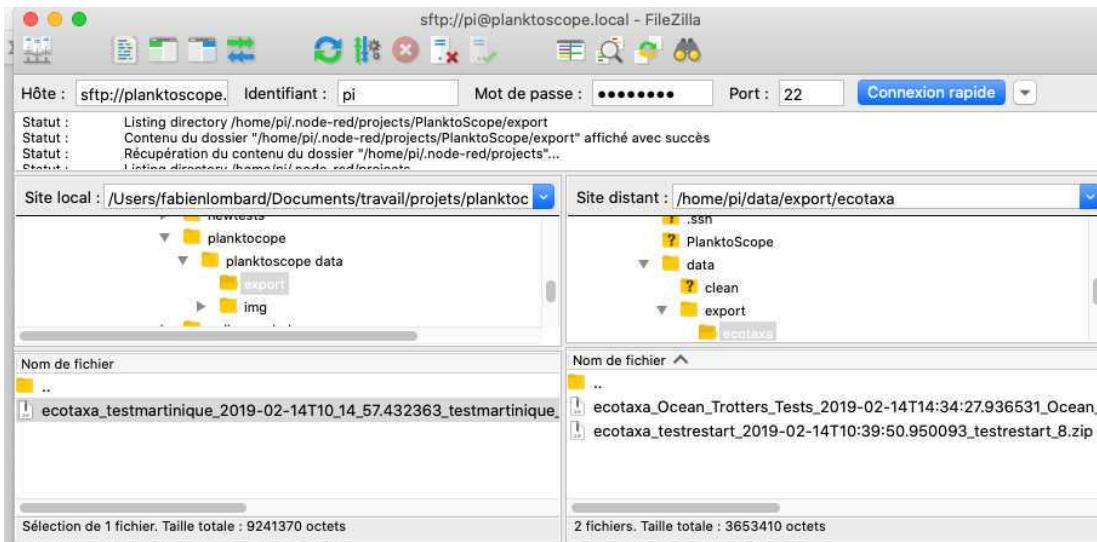
- 7 You will need a computer connected to the planktoscope together with free software FileZilla (<https://filezilla-project.org/>)



- Open FileZilla
- Either clic on the top right to create a new connection or use the quick-connection fields below
 - 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 (on the left) the access to what is in your computer and (on the 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 subtraction, 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 detach 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 more times** until no plankton is visible on the camera)

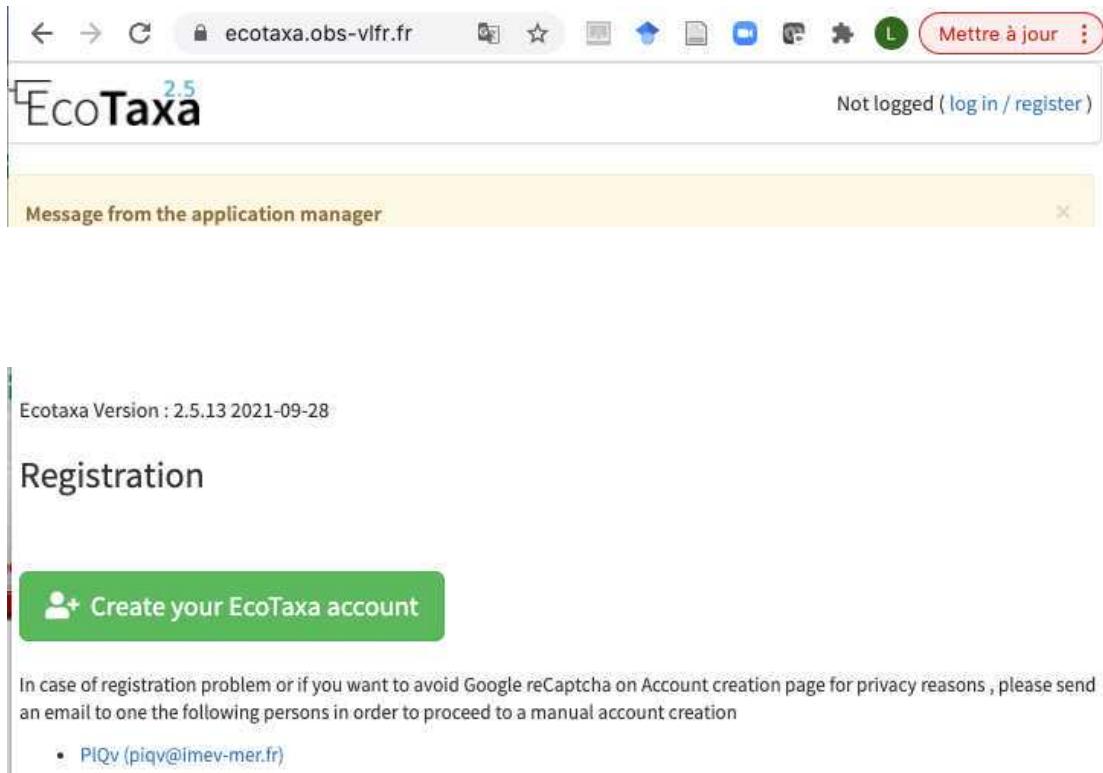
8. Finally drain the system

Upload your images on EcoTaxa

9

At First connection:

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



Put your real name and a valid mail so that you can be contacted

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

Create me

- 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(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 account with the name "test test" and a "(log out)" link. Below it is a dropdown menu labeled "Action". To the right of the account, there is a search bar with a "Search" button. On the left, the title "EcoTaxa 2.5" is displayed. In the center, the heading "Select a project" is shown, followed by a link "How to prepare data". Below this, a message says "To create a new project and upload images in it, please contact the user manager(s):" with an email address "• PIQv (piqv@imev-mer.fr)".

without the right to create projects

The screenshot shows the EcoTaxa 2.5 interface. At the top right, there is a user account with the name "Fabien Lombard" and a "(log out)" link. Below it is a dropdown menu labeled "Action". To the right of the account, there is a search bar with a "Search" button. An error message "Error :2" is visible above the search bar. On the left, the title "EcoTaxa 2.5" is displayed. In the center, the heading "Select a project" is shown, followed by a link "How to prepare data". Below this, there is a green button labeled "Create a new project". To the right, there is a search filter section with fields for "Filter on title", "Exclude subsets", "Instrument", and a "Clear" button.

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

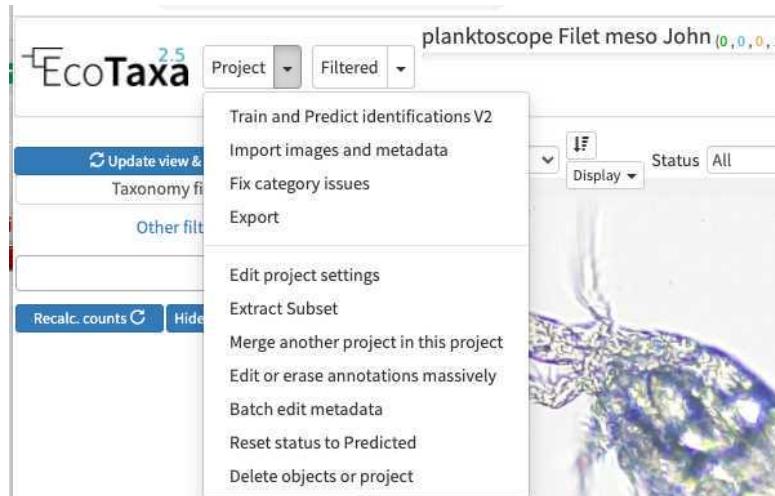
Password : Pl@nkt0n4Ecotaxa

Once this is 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)

Safety information

- Please eventually create your own folder to "try" to keep it clean and tidy
- 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 for one zip file at a time for now)

ECOTaxa 2.5 planktoscope Filet meso John

Images and TSV file import (Step 1)

IMPORTANT NOTE

The maintenance and improvement of EcoTaxa is not aside some funds in your next grant to support it. [Contact us](#) to estimate what would be both reasonable.

How to create a project [?](#)

How to prepare data [?](#)

How to import data from zooprocess [?](#)

Choose a folder or zip file on the server

ftp_plankton/Ecotaxa_Data_to_import/PLANKTONSCOPE/testsept2021

Contact the project manager to know where and how to upload your data. Once the import is complete, all data folders/files can be safely erased on the server.
Project manager : lombard@obs-vlfr.fr

Skip tsv files that have already been imported

Skip objects that have already been imported

Advanced options [?](#)

Upload folder(s) compressed as a zip file

Choisir un fichier Aucun fichier choisi

Only for < 100MB zip files.

Start import Images and TSV files

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

EcoTaxa 2.5 planktoscope Filet meso John

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

Project Title	planktoscope Filet meso John
Data sharing license	<input type="radio"/> 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. <input type="radio"/> 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. <input checked="" type="radio"/> 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. <input type="radio"/> Copyright: only contributors to this project have rights on this data. This prevents its distribution in any kind of database. <input type="radio"/> Not chosen
Visible for all visitors (only validated objects)	<input checked="" type="checkbox"/>
Status	Annotate
Project Description	
Definition of preset for manual sorting	
<input type="button" value="Pick from others projects"/>	
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_flowcam_group	<small>SCN Network used for prediction, if you change it, existing features computed with this network will be erased.</small> <small>New features will be recomputed during the next automatic classification.</small>
-------------	-------------------	--

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

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

10.1 Use filters wisely

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



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

A screenshot of the EcoTaxa interface focusing on the 'Taxonomy filter' tab. The interface shows a hierarchical list of taxonomic groups with counts. The groups listed are: Asterionellopsis (11), Chaetoceros socialis < Chaetoceros (1), Coscinodiscophycidae (47), Bacteriadrum < Chaetoceratales (36), Chaetoceros < Chaetoceratales (41), Dinophyceae (741), Ceratium sp. (644), Neoceratium 01 (601), Neoceratium furca < Neoceratium (108), Neoceratium pentagonum (91), Noctiluca < Noctilucaceae (12), Oxytoxum (245), Pyrocystaceae (2), Pyrophacus (4), Ditylum (75), and Eucampia (2).

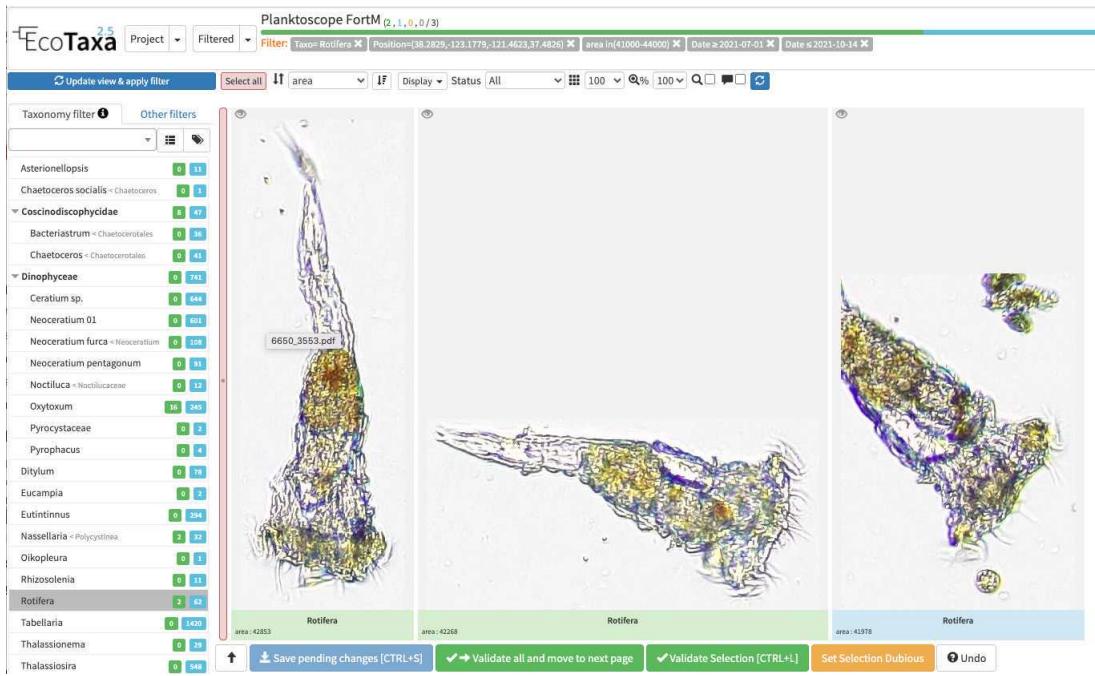
Taxonomic Group	Count
Asterionellopsis	11
Chaetoceros socialis < Chaetoceros	1
Coscinodiscophycidae	47
Bacteriadrum < Chaetoceratales	36
Chaetoceros < Chaetoceratales	41
Dinophyceae	741
Ceratium sp.	644
Neoceratium 01	601
Neoceratium furca < Neoceratium	108
Neoceratium pentagonum	91
Noctiluca < Noctilucaceae	12
Oxytoxum	245
Pyrocystaceae	2
Pyrophacus	4
Ditylum	75
Eucampia	2

taxonomic filters

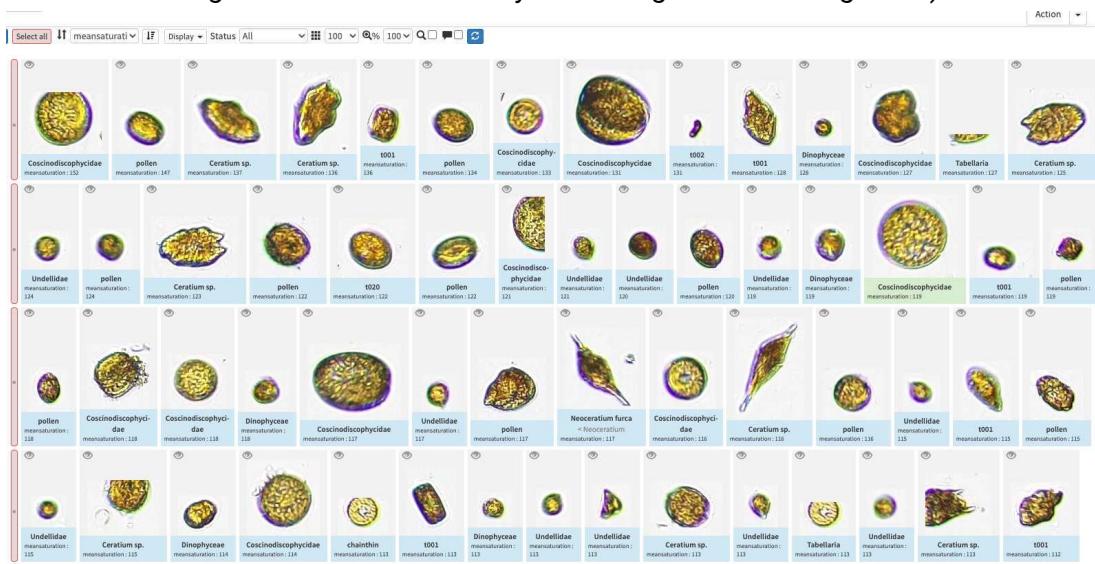
The screenshot shows the EcoTaxa 2.5 filter interface. At the top, there are buttons for 'Project' and 'Filter'. Below that is a blue button labeled 'Update view & apply filter'. A 'Taxonomy filter' section has a 'Share page' button and a 'Clear all filters' button. The 'Sample' section includes a dropdown for 'Sample' and a 'Clear' button. The 'Depth' section allows setting 'Min [m]' and 'Max [m]'. The 'Location' section includes a 'West' dropdown, a 'North' dropdown, a 'South' dropdown, and an 'East' dropdown, with a 'Clear' button. An 'Open map' button is also present. The 'Date' section includes 'Begin', 'End', and 'Month' fields, each with a 'Clear' button. The 'Time' section includes 'Begin' and 'End' fields, a date range slider, and a 'Day time' field. The 'Instrument' section includes a dropdown for 'Instrument' and a 'Clear' button. The 'Annotator / Free filters' section includes a dropdown for 'search user...', a 'Validation date begin' field, a 'Validation date end' field, and a complex filter builder with dropdowns for 'Num. field' and 'Text field' and operators like '>=' and '<=' and 'contains'. At the bottom are three buttons: 'Update view & apply filter' (blue), 'Save filters' (light blue), and 'Apply saved filters and update view' (grey).

other filters

Filters are additive, so you can add filters 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)

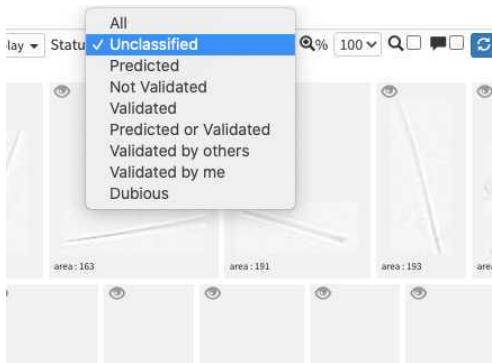


quickfilters are pretty useful since you can sort objects by specific values (eg. mean saturation below) to quickly observe objects that have here lots of chlorophyll (ps. you can revert the sorting order of those filters by ascending or descending order)



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)

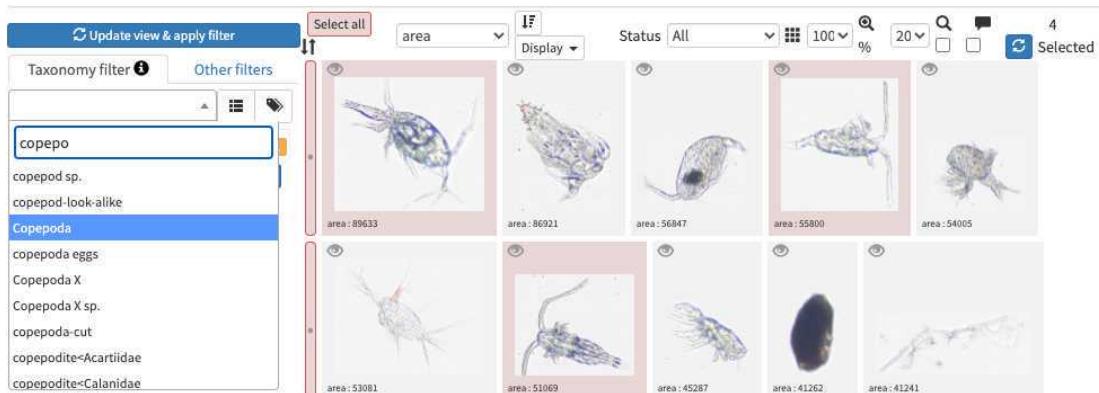


However they could be also set as "predicted" (blue surrounding; classified automatically by taking as example one pre-existing project), "validated" (green surrounding; checked and annotated by a human), or dubious (orange surrounding; checked and annotated as dubious by a human)

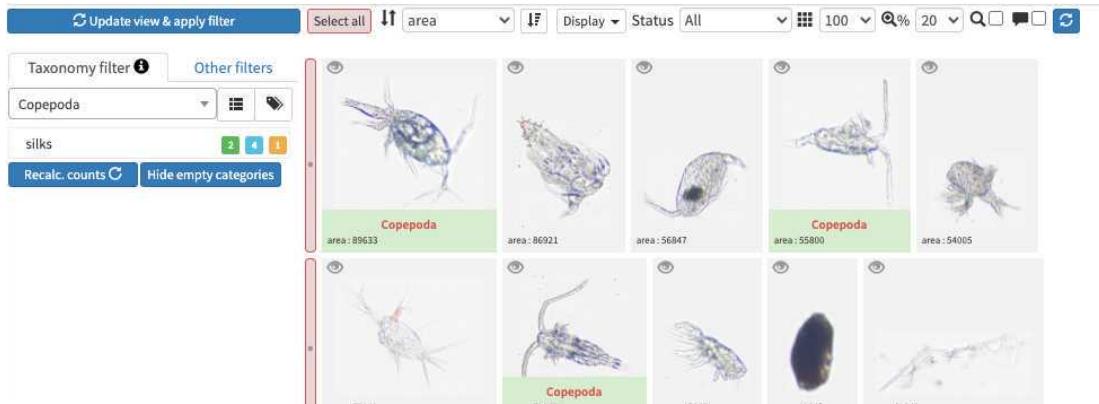


-validating consist in selecting one or several picture and attributing them a taxonomic or morphological identity by either displacing them in the list of taxa present in the « taxonomic filter » tab (in which you can force some categories to be present by using the « preset » in the project settings... 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.

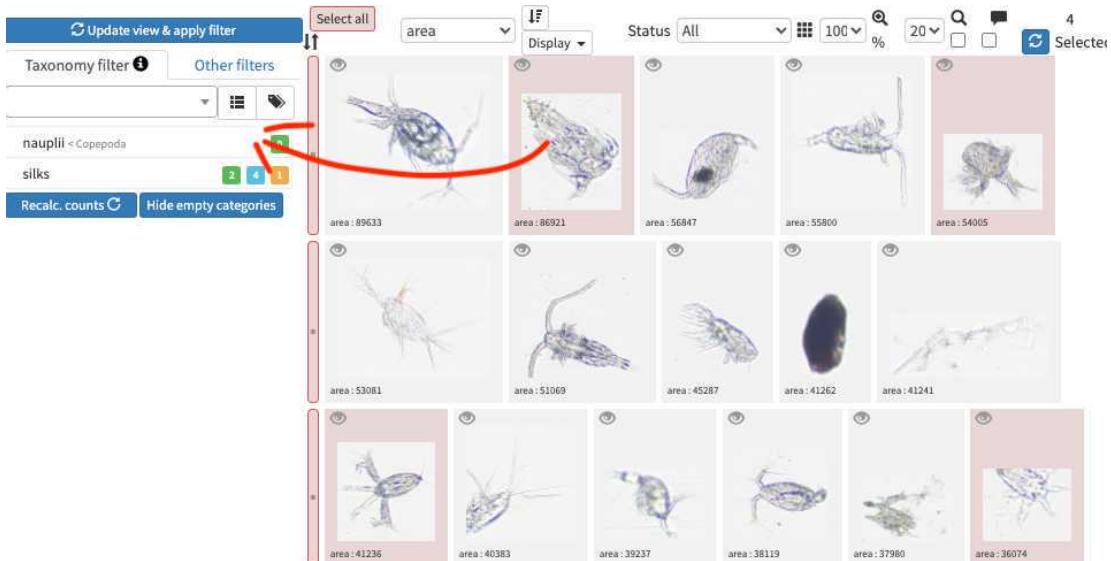
photo here to illustrate



typing "copepo" brings several results



once validated the name appears in red below the images



sliding into existing categories also works



don't forget to save your validations

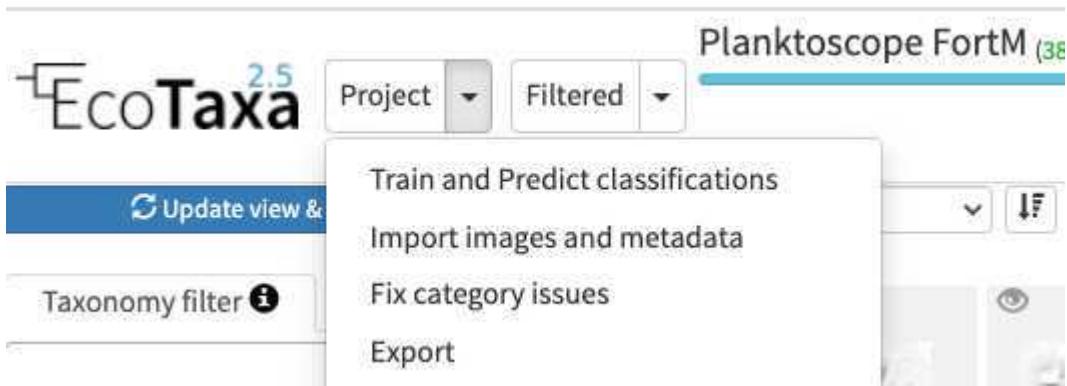
-validation could be tedious and requires large taxonomic expertise, however there are plenty of tools to help you! Filters are one of those tools, but the more interesting one is to use previous project to « predict » some taxonomic identity on your new images, in best cases you will face thousands 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 has nothing to do)

-How to do prediction (and help 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 classifications"



- 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 exist (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

# - Title	# Validated	# Matching features	Deep features model
#4655 - Planktoscope FortM	38	38	flowcam
#4605 - Planktoscope NOAA WCOA21 rita-net	16373	38	flowcam

- Push the button "select project below 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 objects to partly correct the usual strong imbalance between categories (here as an example limited to 100 example per group) (If you use project #4605 as an example, please remove artefacts)

PREDICTION: Choice of Learning Set categories and size

(id)	Source (validated) category	# source	% source	# learning set	Make categories appear like in:
(85008)	artefact	13036	79.4 %	500	Project search
(92010)	silks	998	6.1 %	500	
(85079)	multiple < other	335	2.0 %	335	
(85352)	chainthin	198	1.2 %	198	
(92767)	naupili < Copepoda	174	1.1 %	174	
(89190)	Ceratium sp.	167	1.0 %	167	
(28249)	Ditylum	118	0.7 %	118	
(28117)	Tabellaria	108	0.7 %	108	

- click on "continue 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 relate to position of the vignette in the initial images (bx, by, depth min/max, label, local centroid col/row, x, y)

PREDICTION: Choice of features and settings

[Start prediction task](#)

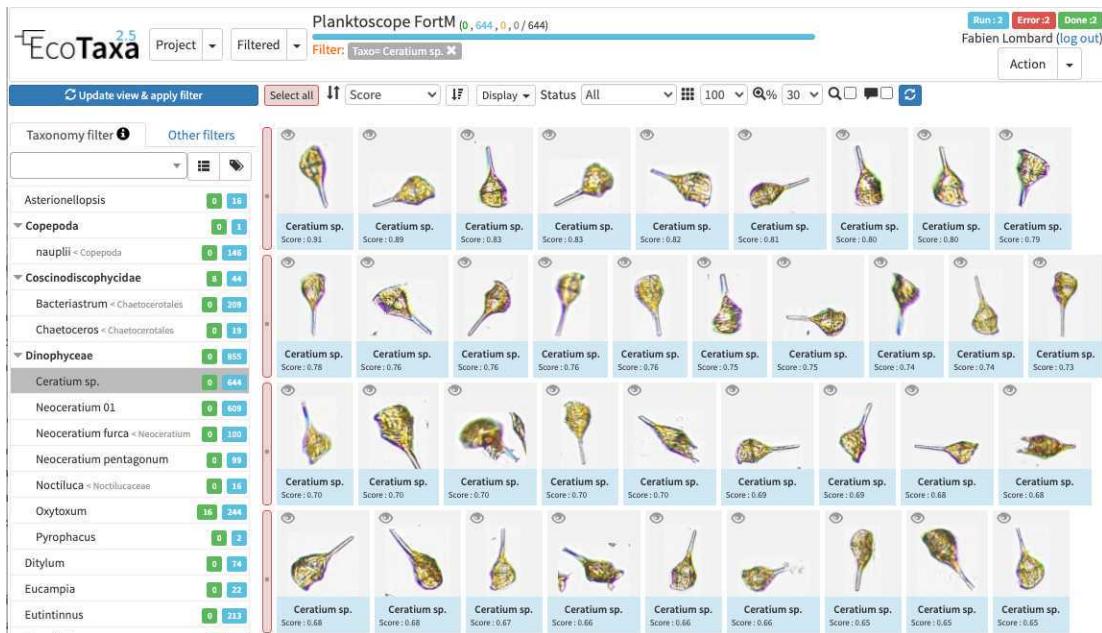
Add deep features

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.

%area <input checked="" type="checkbox"/>	angle <input checked="" type="checkbox"/>	area <input checked="" type="checkbox"/>	area_exc <input checked="" type="checkbox"/>	bounding_box_area <input checked="" type="checkbox"/>	bx <input type="checkbox"/>	by <input type="checkbox"/>
circ. <input checked="" type="checkbox"/>	circex <input checked="" type="checkbox"/>	convex_area <input checked="" type="checkbox"/>	depth_max <input type="checkbox"/>	depth_min <input type="checkbox"/>	eccentricity <input checked="" type="checkbox"/>	elongation <input checked="" type="checkbox"/>
equivalent_diameter <input checked="" type="checkbox"/>	euler_number <input checked="" type="checkbox"/>	extent <input checked="" type="checkbox"/>	height <input checked="" type="checkbox"/>	label <input type="checkbox"/>	local_centroid_col <input type="checkbox"/>	local_centroid_row <input type="checkbox"/>
major <input checked="" type="checkbox"/>	meanhue <input checked="" type="checkbox"/>	meansaturation <input checked="" type="checkbox"/>	meanvalue <input checked="" type="checkbox"/>	minor <input checked="" type="checkbox"/>	perim. <input checked="" type="checkbox"/>	permareaexc <input checked="" type="checkbox"/>
perimajor <input checked="" type="checkbox"/>	solidity <input checked="" type="checkbox"/>	stdhue <input checked="" type="checkbox"/>	stdsaturation <input checked="" type="checkbox"/>	stdvalue <input checked="" type="checkbox"/>	width <input checked="" type="checkbox"/>	x <input type="checkbox"/>
y <input type="checkbox"/>						

Once done, images are now "predicted" (blue) but still wait for validation. Note that while classifying the different objects, the classifier also gives 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)

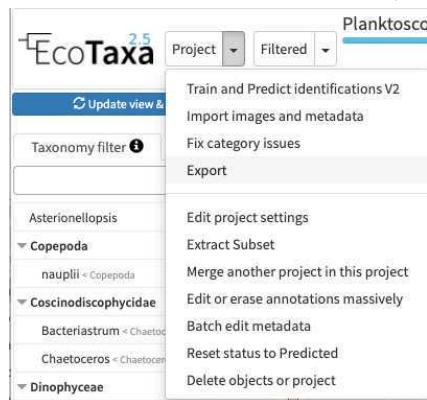


example of images sorted by score of prediction

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 exist, the easiest to understand being the summary export with count per sample)



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 <input type="button" value="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 : <input type="button" value="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 <input type="button" value="Sample"/>

Maintenance of your planktoscope

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 got 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 create a sudden variation of pressure (e.g. ➡ go to step #5.7)
3. Over time, wet conditions and organic matter may create favorable condition for the growth of a bacterial film. The flowcell and tubing will look dirty from the inside. You can avoid this by pumping diluted bleach sometimes, let it act for 1-2 hours and carefully rinse 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 appear as dispersed cristals attached inside the flowcell or a white coating inside the tubing. To remove and clean this, pump some acidic solution (vinegar, citrus juice or other kind of other acids), let it rest for a 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 only dry dusts 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 a 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 go further

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External links

13 Planktoscope website

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

Planktoscope github

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

Planktoscope complete assembly guide and complete documentations

<https://planktonscope.readthedocs.io/en/latest/>

Planktoscope Slack channel (to exchange ideas/protocols/solutions)

<https://forms.gle/qvh5jwuMvmyBKM0C7>

Plankton Planet website

<https://planktonplanet.org/>

EcoTaxa tutorials:

<https://sites.google.com/view/piqv/ecotaxa?authuser=0>

<https://www.youtube.com/watch?v=PS06ZS765tk>

<https://www.youtube.com/watch?v=RaWUqIoKk0E>