

VERSION 3

FEB 22, 2024

OPEN  ACCESS

DOI:

[dx.doi.org/10.17504/protocols.io.
bp2l6bq3zgqe/v3](https://dx.doi.org/10.17504/protocols.io.bp2l6bq3zgqe/v3)

External link:

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

Protocol Citation: Pierre Kostyrka, Lombard Fabien 2024. Planktoscope protocol for plankton imaging. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bp2l6bq3zgqe/v3> Version created by Lombard Fabien

 Planktoscope protocol for plankton imaging V.3Pierre Kostyrka^{1,2}, Lombard Fabien^{1,2}¹Sorbonne Université, Centre National de la Recherche Scientifique, Laboratoire d'Océanographie de Villefranche (LOV), Villefranche-sur-Mer, France;²Institut Universitaire de France, 75231 Paris, France

LOVComplex



Lombard Fabien

DISCLAIMER

This protocol applies to the version 2.5 of the PlanktoScope and the 2.3 version of software. It is optimized to image 20µm-200µm organisms using the 25mm lens (as tube lens) and 12mm one as objective one and may be inaccurate with other configurations or light. Please note that the segmenter is currently also optimized 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 collecting usable results for quantitative imaging of plankton.

This project has received funding from the European Union's Horizon 2020 research and innovation programme "Atlantic Ecosystems Assessment, Forecasting and Sustainability" (AtlantECO GA#862923)

This research was co-funded by the European Union (GA#101059915 - BIOcean5D).

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

IMAGE ATTRIBUTION

Fabien Lombard, Thibaut Pollina, Karine Leblanc, Will Major, Pierre Kostyrka

MANUSCRIPT CITATION:

Pollina T, Larson AG, Lombard F, Li H, Le Guen D, Colin S, de Vargas C, Prakash M (2022) PlanktoScope: Affordable Modular Quantitative Imaging Platform for Citizen Oceanography. *Frontiers in Marine Science* 9. doi: 10.3389/fmars.2022.949428

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

Mériguet Z, Oddone A, Le Guen D, Pollina T, Bazile R, Moulin C, Troublé R, Prakash M, de Vargas C, Lombard F (2022) Basin-Scale Underway Quantitative Survey of Surface Microplankton Using Affordable Collection and Imaging Tools Deployed From Tara. *Frontiers in Marine Science* 9. doi: 10.3389/fmars.2022.916025

de Vargas C, Le Bescot N, Pollina T, Henry N, Romac S, Colin S, Haëntjens N, Carmichael M, Berger C, Le Guen D, Decelle J, Mahé F, Poulain J, Malpot E, Beaumont C, Hardy M, Guiffant D, Probert I, Gruber DF, Allen AE, Gorsky G, Follows MJ, Pochon X, Troublé R, Cael BB, Lombard F, Boss E, Prakash M, the Plankton Planet core team, Bazile R, Boss E, Bourdin G, Cael B, Casati R, Colin S, Vargas C de, Gorsky G, Guiffant D, Haentjens N, Henry N, Larson A, Bescot NL, Lombard F, Mirambeau G, Moulin C, Oddone A, Prakash M, Prazuck C, Raimbault V, Trellu C, Troublé R (2022) Plankton Planet: A frugal, cooperative measure of aquatic life at the planetary scale. *Frontiers in Marine Science* 9

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 component 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://planktonscope.readthedocs.io/en/latest/>

MATERIALS

- Plankton net or other kind of microplankton collector
- 200µm sieve
- Squizing bottle
- Micrometer slide (or millimetric ruler)
- Optical paper
- Dry gas dispenser
- PlanktoScope box
- Fine forceps
- A computer

Softwares :

- ImageJ (last version, needs to compute RGB images)
- FileZilla
- BalenaEtcher

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol and it's working

Created: Feb 21, 2024

Last Modified: Feb 22, 2024

PROTOCOL integer ID: 95546

Keywords: Planktoscope, plankton, microscopy, quantitative imaging, microplankton

Funders Acknowledgement:
This project has received funding from the European Union's Horizon 2020 research and innovation programme "Atlantic Ecosystems Assessment, Forecasting and Sustainability" (AtlantECO)
Grant ID: 862923
This research was co-funded by the European Union (GA#101059915 - Biocean5D).
Grant ID: 101059915

SAFETY WARNINGS

- ! Planktoscope is an electronic device, powered with electricity. It is therefore sensible 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" contained 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)

- For an easiest navigation, you can see the table of contents by clicking on "Show the table of contents" on the top left.
- Figures are numbered by section:
If a figure is cited in its corresponding section, only the number will be cited (ex: fig.1).
If a figure is cited in a different section, the section and the number will be cited (ex: S3-fig.1 for figure 1 of section 3).

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

Table of Contents

1. Table of Contents  [go to step #1](#)
2. Introduction  [go to step #2](#)
3. Quick usage version  [go to step #3](#)
4. Assemble the PlanktoScope  [go to step #4](#)
5. User Interface and initial connexion  [go to step #5](#)
6. How to export data  [go to step #6](#)

7. White Balance calibration  [go to step #7](#)
8. Pump calibration  [go to step #8](#)
9. Size calibration  [go to step #9](#)
10. Get your sample  [go to step #10](#)
11. Pass the sample on the PlanktoScope  [go to step #11](#)
12. Segment the Acquisition  [go to step #12](#)
13. Clean the PlanktoScope  [go to step #13](#)
14. Upload your images on EcoTaxa  [go to step #14](#)
15. How to use efficiently EcoTaxa  [go to step #15](#)
16. How to compute biovolumes  [go to step #16](#)
17. Maintenance of your PlanktoScope  [go to step #17](#)
18. Update the software or reset the PlanktoScope  [go to step #18](#)
19. Troubleshooting  [go to step #19](#)
20. External links  [go to step #20](#)

Introduction

2 The **PlanktoScope** is a frugal, microfluidic microscope designed with an open-hardware and open-software approach. It was conceived with the idea of providing the thousands of scientists and sailors exploring the oceans with a high-quality instrument suitable for deepening our knowledge of the sea around us. In this manual you will learn how to operate the Planktoscope and take images of plankton.

Quick usage version

3 This part is a quick version of the protocol, where you will find the essential steps to follow. **If you're using your PlanktoScope for the first time or need to calibrate it, please read the full protocol. Don't wait too long between the sampling and the processing of your sample through the PlanktoScope to avoid sedimentation and aggregation.**

3.1 Preparation

1. Assemble and plug in the PlanktoScope  [go to step #4](#)
2. Connect to PlanktoScope's Wi-Fi  [go to step #5.1](#)

3. Go to <http://192.168.4.1:1880/ui/> (v2023: <http://home.pkscope/> > Node-RED Dashboard)
4. Fill ALL the sample details (in SAMPLE) [Critical] [➡ go to step #11.1](#)
5. Put the light on (in OPTIC CONFIGURATION)
6. Check the WB parameters (*you should have already done it during the initial connection*)
[➡ go to step #7](#)
7. Check flow cell alignment and check the focus [➡ go to step #11.2](#) [➡ go to step #11.3](#)
8. Put 20 mL sample [➡ go to step #11.4](#)
9. Turn on the bubbler
10. Check the focus (in OPTIC CONFIGURATION) [➡ go to step #11.3](#)

3.2 Acquisition

1. Fill acquisition parameters (in FLUIDIC ACQUISITION) [➡ go to step #11.7](#)
2. Pump to drain sedimented organisms before acquisition (in OPTIC CONFIGURATION)
3. Start the acquisition (in FLUIDIC ACQUISITION)

3.3 Segmentation and data export

1. Fill in segmentation parameters (in SEGMENTATION) [➡ go to step #12](#)
2. Start segmentation
3. Back-up data (in GALLERY or by using FileZilla) [➡ go to step #14](#)
4. Import on Ecotaxa [➡ go to step #14](#)

3.4 Cleaning [➡ go to step #13](#)

1. Drain the syringe (disconnect your system)
2. Drain the content
3. Replace with fresh water and drain several times (blowing in the syringe may helps)
4. Replace the system and drain first with tap water and then air
5. Empty waste container

If not used immediately

1. Put 20 mL diluted bleach
2. Leave 15'
3. Drain the content (high pump speed)

4. Put 10 mL fresh water
5. Drain the content (high pump speed)

3.5 Shut down

1. Turn OFF virtually (in HOME) and wait one minute
2. Unplug the PlanktoScope

Assemble the PlanktoScope

4 The PlanktoScope kit

In this part you will learn :

1. What is inside the PlanktoScope kit
2. How to assemble the fluidic path with the flowcell
3. How to assemble the bubbler
4. How to assemble all the components together

Open the PlanktoScope box and check that everything is in it. The PlanktoScope kit also includes a bubbler, power cable, waste container, falcon tube of tap water, syringe, sample holder, flowcell holder and flowcell (fig.1; fig.2).

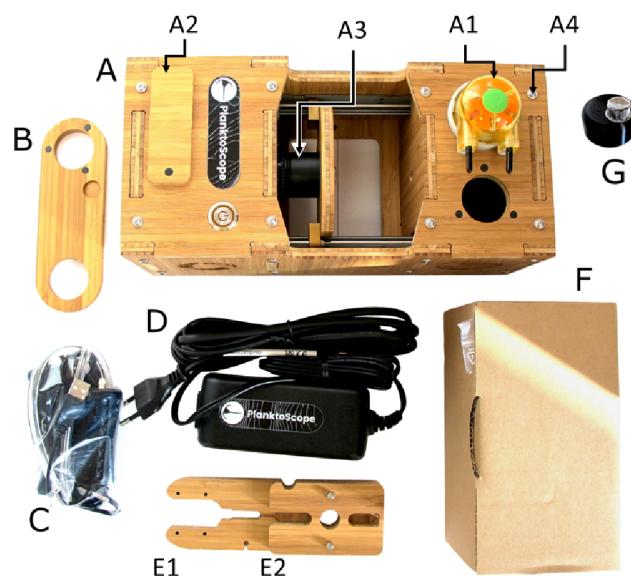


Figure 1 : The PlanktoScope Kit. (A) The main box with the pump A1, the USB cover A2, the pi camera A3 and the screws A4; (B) Tube holder; (C) Bubbler; (D) Power cable; (E) Flow cell receptacle; (F) Supplementary materials box; (G) Camera cover to protect the camera. *In the box F, you can find : A 200um filter; an eyedropper; a SD card adaptor and a screwdriver; flow cells; cleaning kit and all the fluidic circuit material.*

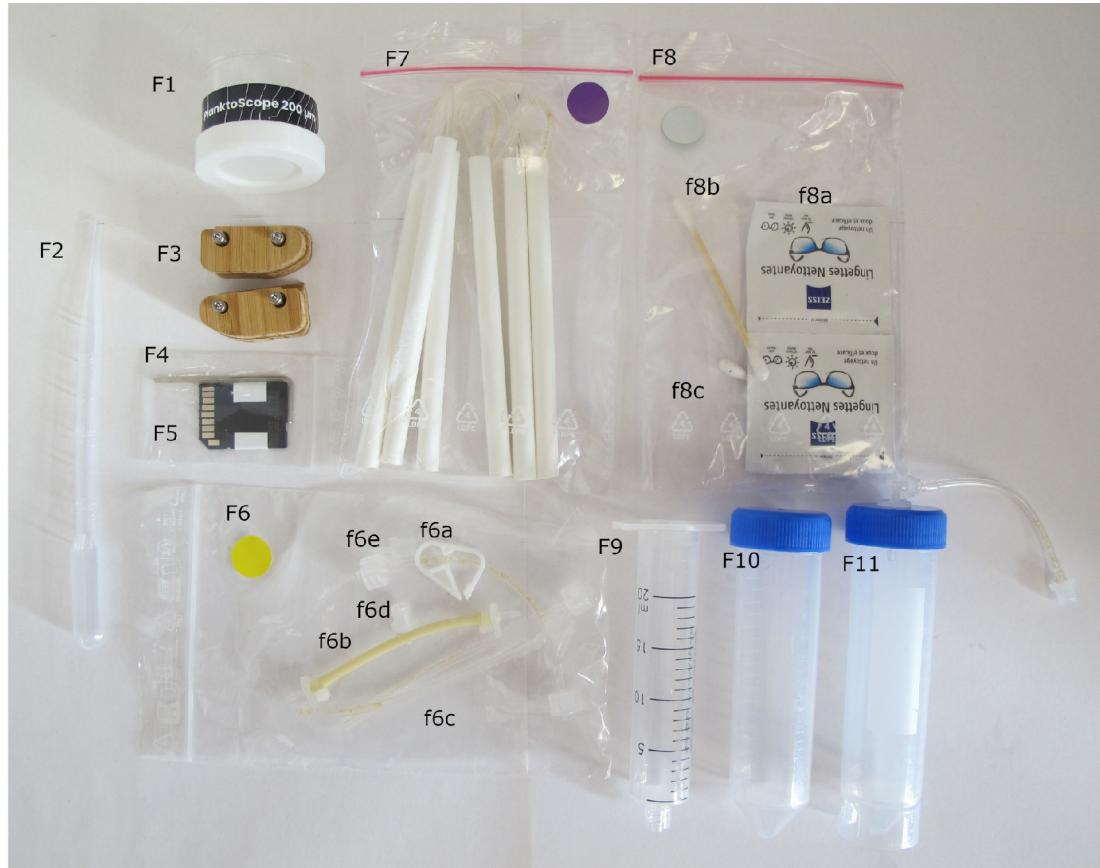


Figure 2 : Material inside the box F. (F1) 200um filter; (F2) eyedropper; (F3) syringe holders; (F4) screwdriver; (F5) SD card adaptator; (F6) fluidic path; (f6a) Clip to stop the flow; (f6b) internal pump tube duplicate; (f6c) tubes and clips for the fluidic path; (F7) flow cells; (F8) cleaning kit; (F9) syringe; (F10) sample tube; (F11) trash tube.

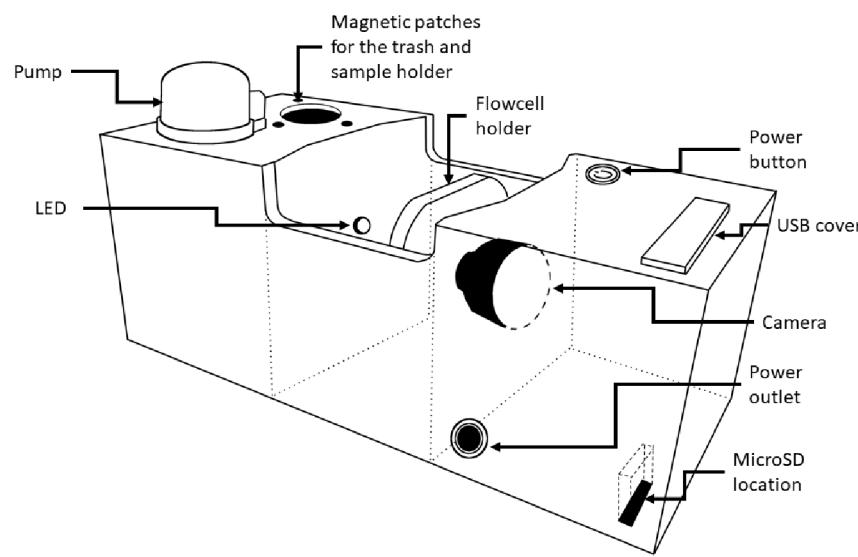


Figure 3 : Main components of the PlanktoScope box (A in fig.1).

Safety information

If it is present, don't forget to remove the **transparent cover** from the camera (fig.4).



Figure 4 : The transparent cover of the camera.

4.1 The flow cell

Safety information

The flow cell can break easily. It is a part to handle with care. There is multiple flow cell in the box to replace broken ones.

- Do not touch it with your fingers or leave it on a surface.
- If a flow cell is dirty, you can clean it softly with the cleaning kit.
- **The flow cell receptacle should be placed with caution on the lens to not break the flow cell. It will clip with the magnets.**
- Do not stretch the tubes attached to the flowcell, as this may damage the flowcell.

1. It is recommended to remove the upper screwed parts used to maintain the syringe (F3 in fig.2).
2. Take a flowcell and plug the short part to the syringe with the help of a male clip.
3. Install it like in the image on the left of the fig.5.
4. Then, press the tube where the arrows point (fig.5).
5. Screw softly the two parts together.
6. Place the receptacle on the magnetic flowcell holder. You can put some tape on the magnetic parts to reduce the "impact" between the flowcell holder and the receptacle.



Figure 5 : How to install the flow cell. You will need the syringe (F9), the flowcell receptacle (E) and one flowcell (F7).

4.2 The fluidic path

Now that you have fixed the flowcell (see last section), assemble all the fluidic system as below (fig.6). Don't forget to install the "tube clip" on the matching tube (figure below). It will allow you to stop the flow when it is necessary.

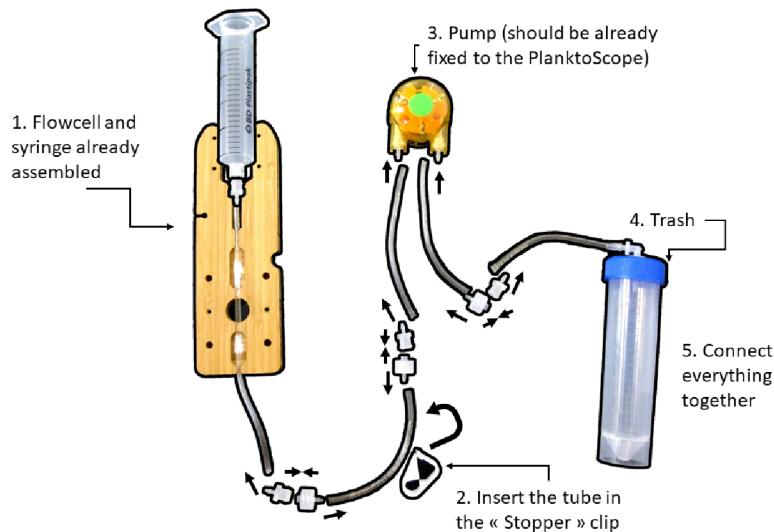


Figure 6 : How to assemble the fluidic path.

4.3 The bubbler

Planktoscope image fluid at low speed. You will need a "bubbler" to avoid sedimentation.

Safety information

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 overestimate true concentrations.

1. Assemble the bubbler like in the image below (fig.7). **It is recommended to use a cut glass pipette or something similar instead of the needle** provided (the needle can be clogged and does not allow a good control on the air flux).
2. Plug the Bubbler into one of the USB ports on the PlanktoScope.
3. Place the tubing into the syringe so that it reaches the bottom.
4. Affix the tubing to the syringe using an elastic band, string or similar.
5. Setup the 'Bubbler': the flow of air needs to be adjusted to 1 bubble/sec approximately, bubbling the bottom of the syringe. You will need water to check this.
6. Do not put the tip of the bubbler in the center of the syringe or it will inject bubbles into the flowcell.

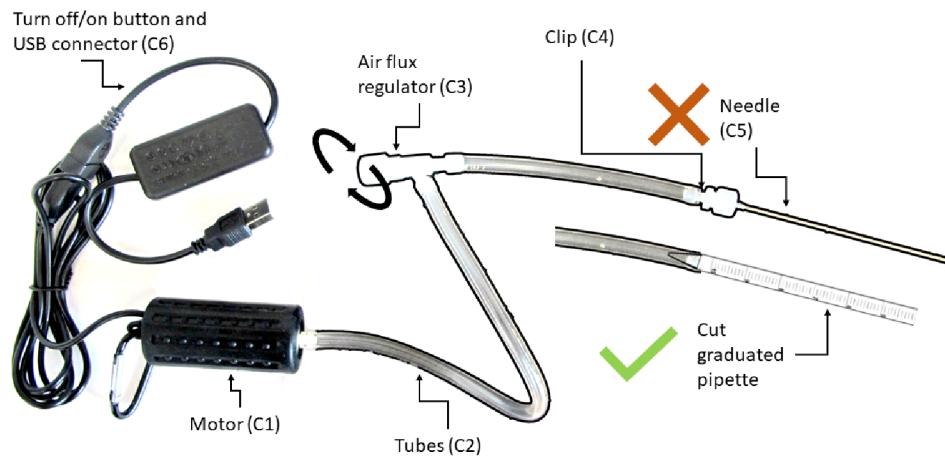


Figure 7 : The bubbler.

4.4 Assemble the PlanktoScope

The fluidic path, the flowcell and the bubbler should be all assembled now.

Now you just need to (if not already done) :

1. Place the flask holder on the magnetic patches (B in fig.1)
2. Place the trash in the outer hole of the flask holder.
3. Place the sample tube in the inner hole.
4. Place the flowcell receptacle on the magnetic flowcell holder  go to step #4.1
5. Plug the bubbler  go to step #4.3
6. Plug the power cable

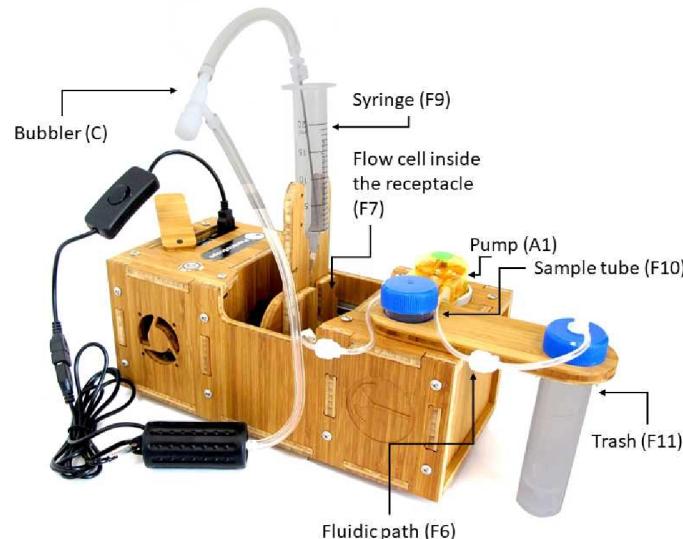


Figure 8 : Assembled PlanktoScope.

User Interface and initial connexion

5 Initial connection

You can access to the user interface of the PlanktoScope by connecting to the Wi-Fi generated by the PlanktoScope. Your computer will be only a projection of the software of the PlanktoScope. It means that even if you disconnect from the Wi-Fi of the PlanktoScope, it will still be running.

1. Power your Planktoscope by connecting the power cable to the power input and turning on the wall switch.
2. Within 1 minute of turning on your PlanktoScope, you should see the LED flash once. This means that the PlanktoScope is ready to be connected by Wi-Fi.
3. You should see a new option for Wi-fi appearing on your computer. Connect to it with the password: "copepode".

For more information and alternative methods of connection, see the designer's Connectivity Tutorial

 PlanktoScope - Connectivity Tutorial (1)
here:

Note

Note that when you are connected to the Wi-Fi of the PlanktoScope, you cannot access to the internet. If you want to use internet at the same time, you should use another computer or an ethernet cable.

5.1 The User Interface (Node-RED dashboard, UI)

Open the PlanktoScope's User Interface (UI) on your web browser (Chrome, Firefox, Edge etc.) using the following webpage link (either click on the link or copy and paste into your browser) and go to the Node-RED dashboard :

<http://192.168.4.1:1880/ui/> (v.2022)

<http://home.pkscope/> > Node-RED dashboard (v.2023)

There are several tabs on the User Interface (UI, fig.1) that can be used to adjust setting, run samples and take images. To navigate around the UI, all tabs are available from the Home tab, including the Shutdown button which we will use when we have finished using the PlanktoScope. We can also use the '**Hamburger Menu**', situated in the top-left corner of the UI, to navigate between tabs.

Safety information

v.2023:

Apart from the Node-RED Dashboard, you can also access to the data file manager, the protocol for the PlanktoScope, or the log in case of errors.

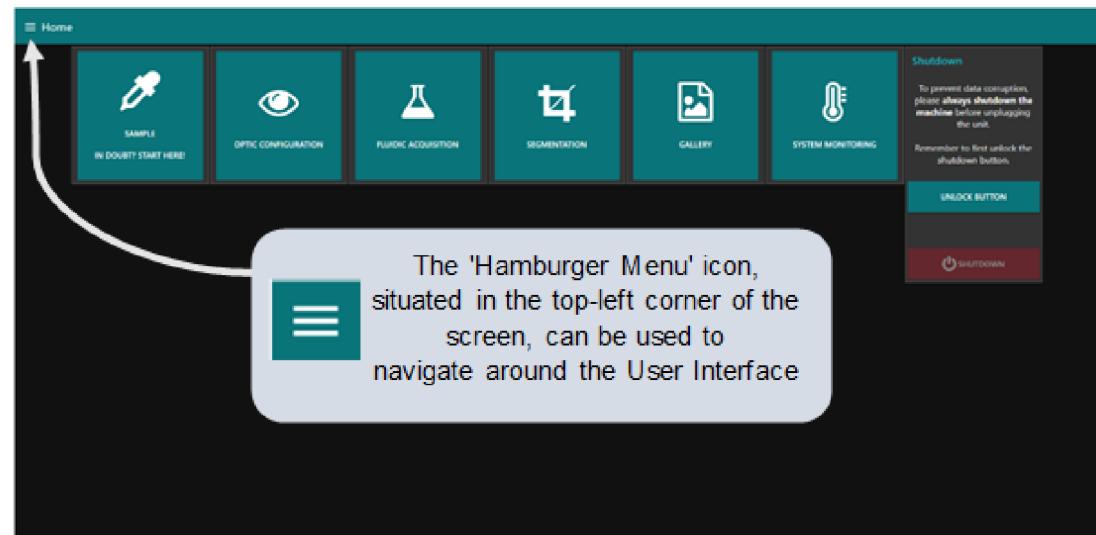


Figure 1: The 'Home' tab of PlanktoScope's User Interface. The 'Hamburger Menu' icon, situated in the top-left corner of the screen, can be used to navigate around the User Interface. **You can adjust the 'Zoom' on your browser (usually Ctrl + scroll UP or DOWN on Windows or command + scroll UP or DOWN on Mac).**

You will find :

1. The "SAMPLE" page, to fill all the metadata of your sample.
2. The "OPTIC CONFIGURATION" page allows you to control the various features of PlanktoScope. You can focus, turn on the LED or start the pump.
3. The "SEGMENTATION" page is used to start the segmentation of the images taken in the previous phase. The images will then be processed to extract only the plankton thumbnails.
4. In the "GALLERY" you can find the different files of the Planktoscope: the exports for EcoTaxa, the original images and the extracted thumbnails.
5. The "SYSTEM MONITORING" page allows you to check the correct operation of the device.
You will not use this step in standard use.
6. The "WIFI" page gives you access to the characteristics of the wifi generated by the PlanktoScope to which you will connect in order to control the device. You will not have to modify anything on this page. The procedure for connecting will be detailed later in this manual.
7. The "HARDWARE SETTINGS" page. You will not need it and it is strongly advised to not change anything else.

5.2 The Optic Configuration tab

Once the UI has loaded on your browser, navigate to the **Optic Configuration** tab and we will make sure the PlanktoScope is operating correctly (fig.2).

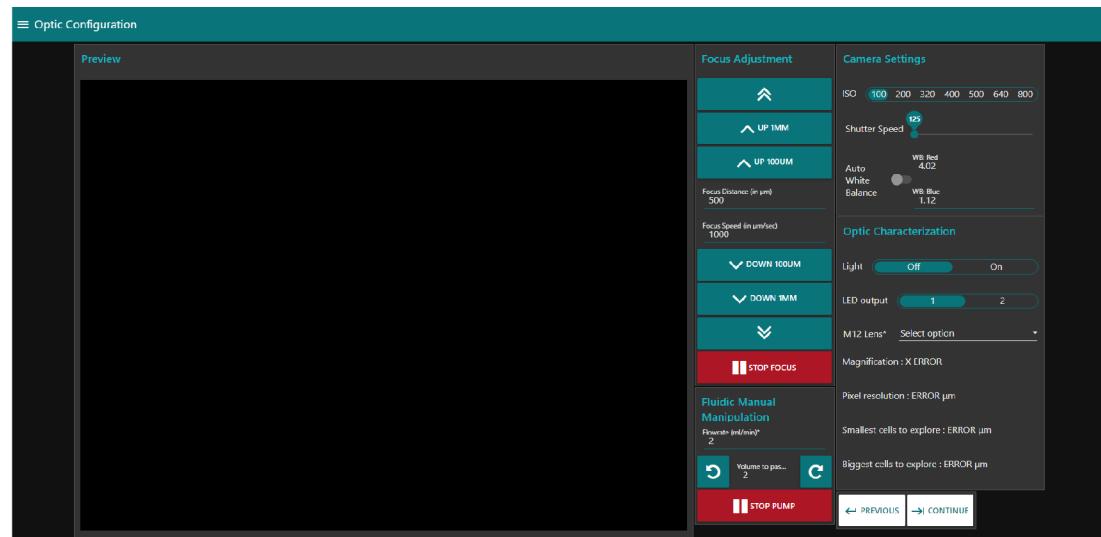


Figure 2: The Optic Configuration tab which can be used to adjust the camera settings. If only Preview is visible on your screen, the other options should be available below by scrolling down.

a) Under Optic Characterisation, **switch on the Light** by clicking 'On' (fig.3). You should see the Preview image turning from dark to light. *The Preview image could be any color so do not worry if yours show blue, red, green, etc. ; it will be adjusted later during the White Balance (WB) calibration.*

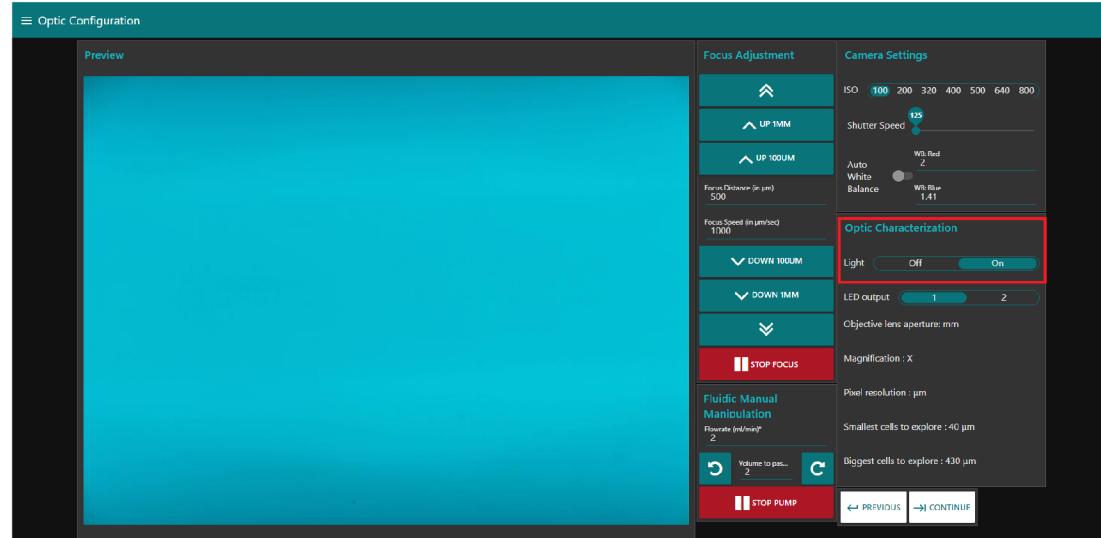


Figure 3: The red box highlights the location for turning on the LED. **You will need to do this every time you use your PlanktoScope.**

b) Under Focus Adjustment (fig.4), **click 'UP 1MM' and 'DOWN 1MM'** to ensure focus buttons turn the focus motor (same for the 100 microns version of the button). You should see the

mount moving further from (UP) or closer to (DOWN) the camera.

You can also use the ">>" button to move your focus according to a personalized value ("Focus distance").

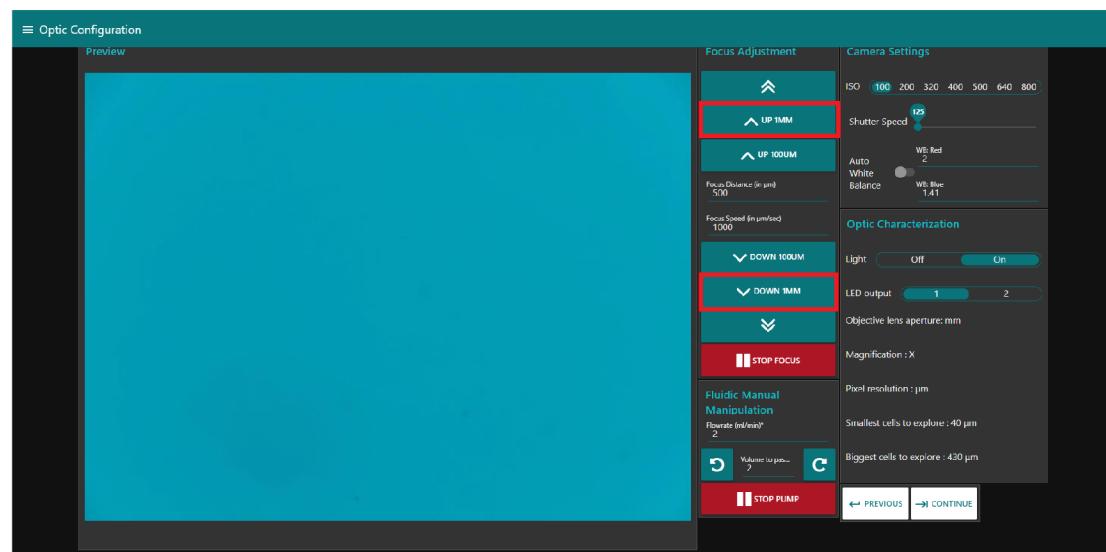


Figure 4: Red boxes highlight 'UP 1MM' and 'DOWN 1MM' that will move the Mount (pictured below).

c) Under Fluidic Manual Manipulation (fig.5), **click clockwise arrow** to check that the Peristaltic Pump is working. You should see the pump rotating in an clockwise direction. You can choose to pass a volume, but keep in mind that **this parameter will not impact the acquisition**. On the other hand, the flowrate parameter will also be effective during an acquisition.

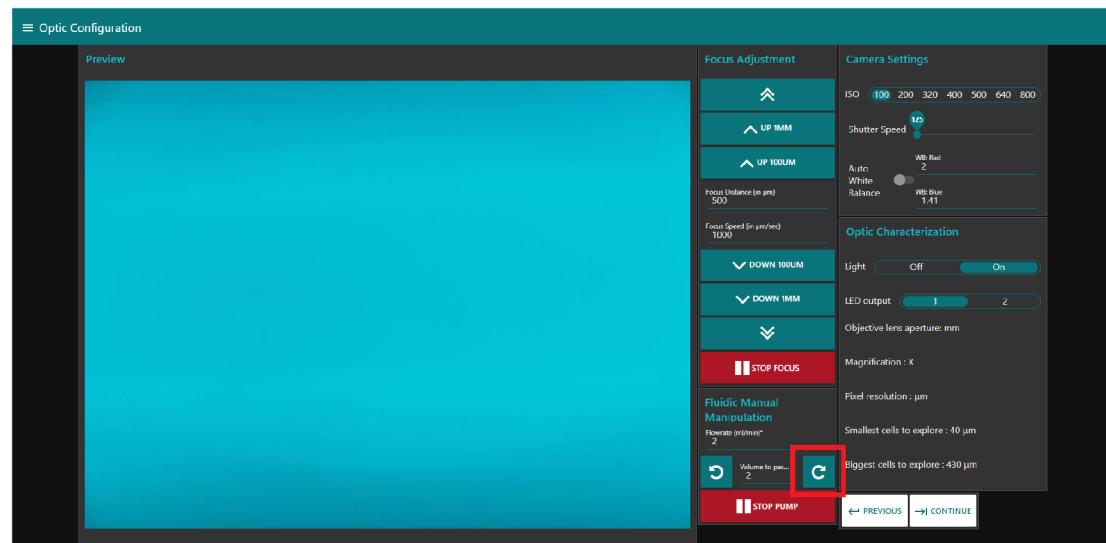


Figure 5: The red square highlights the location of the clockwise arrow that will rotate your Peristaltic Pump in the same direction.

d) Under Camera Settings, you can see a button to change the ISO value (fig.6). **Set the ISO to 100.**

The ISO is the light sensitivity of the camera. A low ISO will be less sensitive to light and then darker. A low ISO tends to provide images of better quality.

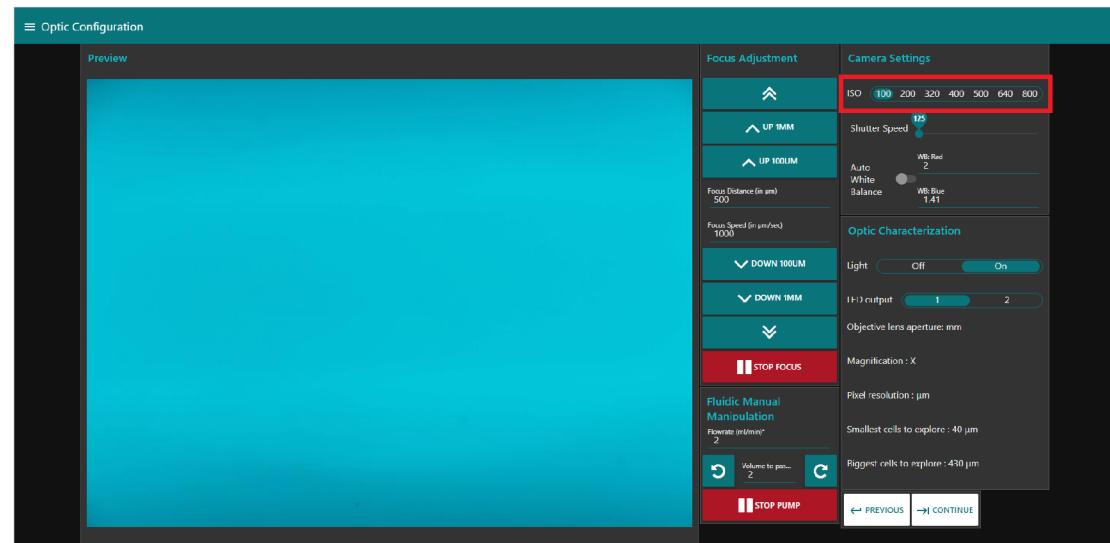


Figure 6: The red box highlights the location of the ISO setting. You should see your Preview image change color when you adjust this setting.

Safety information

Keep the shutter speed at the minimal value. The shutter speed determine how long the light will enter the camera to take one image. A low shutter speed provides better quality, but it also requires the objects to not move too fast.

How to export data

6

Safety information

In v.2023, you can now use the datafile manager (data > export) to download your files directly. In both versions, you can also use the gallery and directly download the images. It is then possible to no longer use FileZilla.

With Filezilla

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

1. Open FileZilla
2. Either click on the top left to create a new connexion or use the quick-connection fields below (it will not save the ftp site for later, fig.1)
3. To create a new connexion "file > site manager > new site"

Enter the following information:

- Host: sftp://192.168.4.1 (note images were taken with a previous version, the address does not correspond to images)
- Username: pi
- Password: copepode
- Port: 22



Figure 1: How to log to a ftp host.

1. Click on connect
2. 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 (click and slide to transfer data in between both)

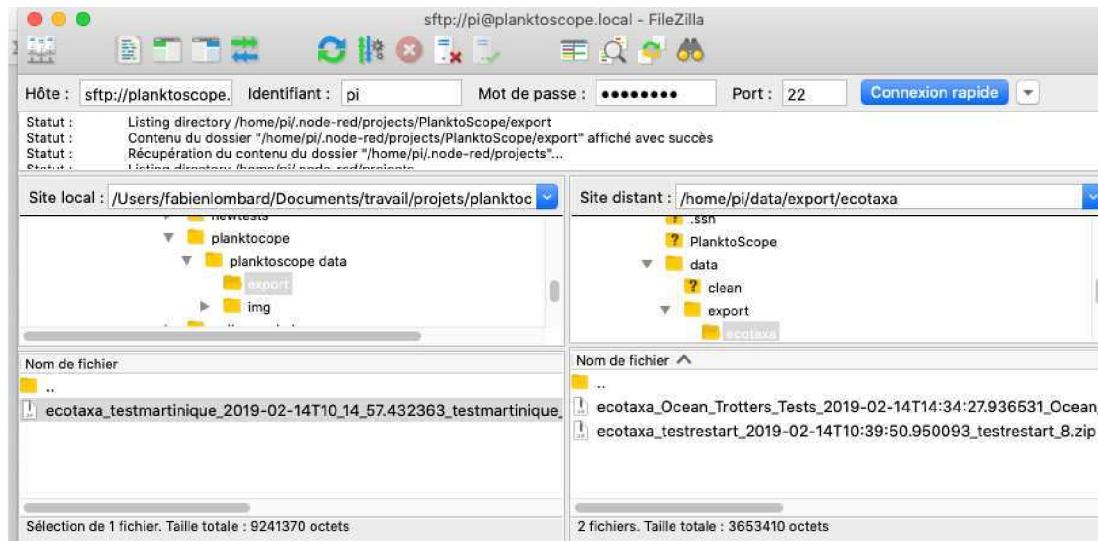


Figure 2: Interface of filezilla. Personal files are on the left, files of the ftp host are on the right.

- 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**

With the gallery

Go to the Gallery tab (fig.3):

- For raw images: **img**
- For segmented images used in EcoTaxa: **export > ecotaxa**
- For detailed process: **clean**
- For segmented images without metadata: **objects**

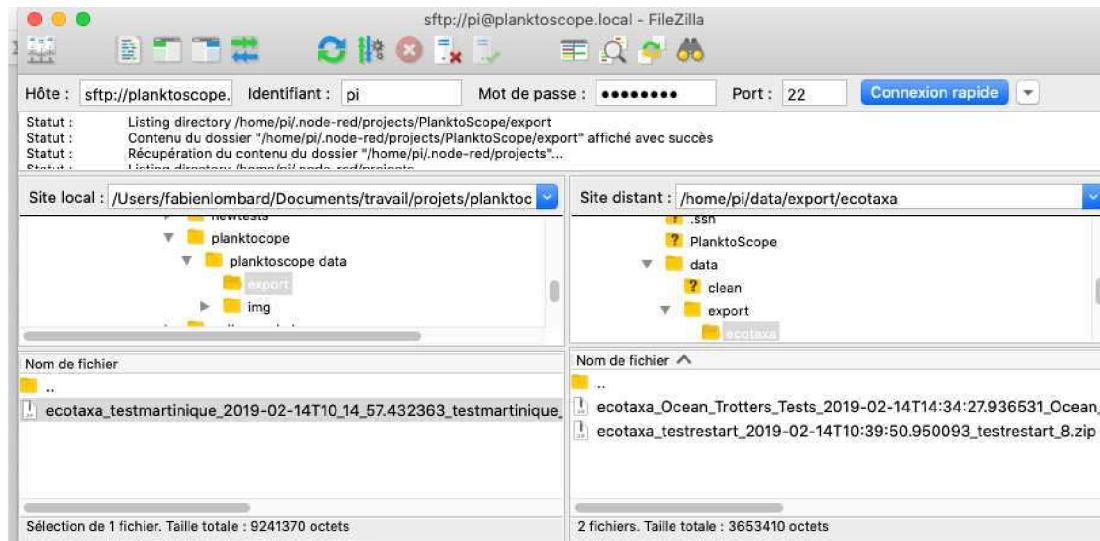


Figure 3: User interface of the gallery tab.

White Balance calibration

7 White Balance (WB) calibration

Stay in the **Optic Configuration tab**. For this step, you will need the software **ImageJ** <https://imagej.nih.gov/ij/index.html>. To check the WB, do not put the flowcell or anything between the camera and the LED.

Note

To calibrate the PlanktoScope or if you just need to do a test, you can use the "**Test Mode**" in the "SAMPLE" tab. It allows you to not fill the date or the location of the sample.

Safety information

Planktoscope are normally cross-calibrated for white balance initially, this information could be recovered from the provider. **We strongly encourage you to note the initial values** before trying to change those and this procedure should not be done without reasons (incorrect image with initial calibration; reboot or update of the software).

Note your initial calibration here:

WB Red:

WB Blue:

Try pressing the Auto White Balance button to its 'on' and 'off' positions on the Optic Configuration tab; you will likely see the Preview image changing color (fig.1).

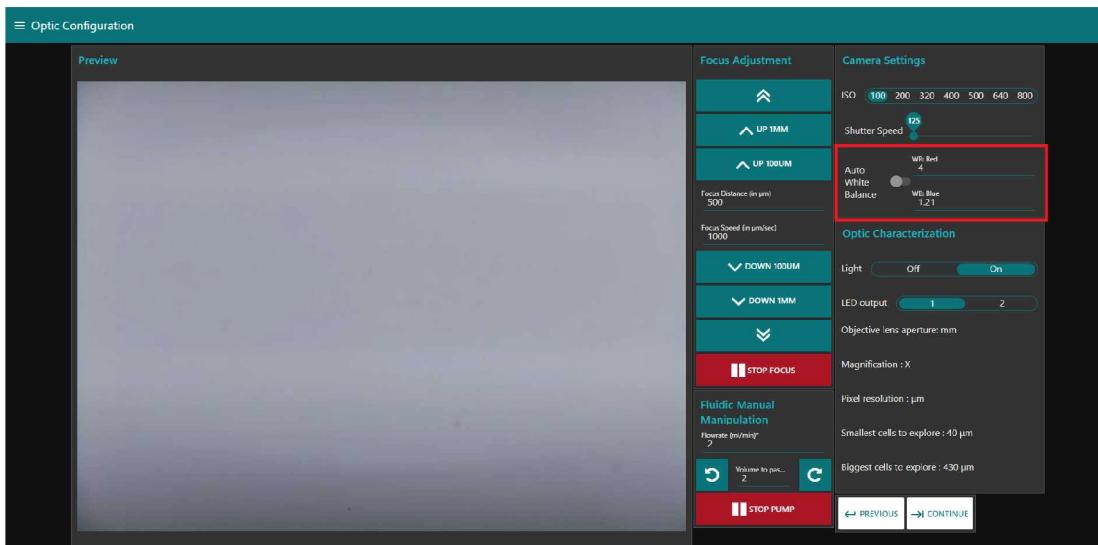


Figure 1 : The red box highlights how to manually adjust the white balance of the Preview image. In this example, the correct setting was WB: Red = 4 and WB: Blue = 1.21. The AWB button should be set to 'off' once you have completed this step.

The Auto White Balance feature is currently not optimized. In addition, not using Auto White Balance enhances the performance of the PlanktoScope over time (the camera will try to adjust it in between every image). **It is then recommended to manually adjust the white balance of your PlanktoScope.** Set the AWB button to 'off'.

To manually set to the White Balance:

1. Turn off the Auto White Balance. You will need to adjust WB: Red and WB: Blue until it looks white/grey. The WB should never be perfect white (but grey), as it imply over-exposition during the segmentation step. **Never set it to 0 or less, or it will create a bug in the software.**
2. Take one image in the acquisition tab (you should not use the preview image as it will not show the real colors taken by the camera).
3. Download the raw image in the "gallery" tab or by using filezilla.
4. Open the software **ImageJ**. The corrected WB Red or Blue is the ratio between the green value (G) on the corresponding color value (Red = R ; Blue = B) multiplied by the old configuration. You can extract the RGB mean values on a software like ImageJ.
5. File > open.
6. Plugins > analyze > RGB. If you do not have the plugin or if it is not working go to *Analyze > Histogram > Click on RGB* until you see the mean red, green and blue values.
7. Once the values are extracted, calculate the ratio G/R and G/B like in the example below.
8. multiply it by the old one to get the new one.

9. Change the configuration.

Example :

If the default configuration is:

$$WB_{red}^{old} = 1$$

$$WB_{blue}^{old} = 2$$

With this actual configuration, we measure the RGB values:

$$R = 245, G = 240, B = 230$$

$$G/R = 0.98$$

$$G/B = 1.04$$

So the new configuration should be:

$$WB_{red}^{new} = WB_{red}^{old} * G/R = 1 * 0.98 = 0.98$$

$$WB_{blue}^{new} = WB_{blue}^{old} * G/B = 2 * 1.04 = 2.08$$

Note

The green color is a fixed value. It is then a way to check the exposure of your image. Because the camera changes the exposure between the preview and the acquisition, it is recommended to **check the green value with an acquisition** and not with the preview. **It should never be at 255.**

If the light exposure is too important (>250), put some tape on the LED to decrease it, and do the WB calibration again.

Also, if the exposure is too important, it will be impossible to correct the WB.

Pump calibration

8 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).

8.1 Calculate pump step

1. Prepare a large volume of tap water and put in in the syringe targeting a total volume A of 20ml
2. On the optic configuration tab: choose to pass a volume Y = 10 mL and record the exact volume X it finally ends to pass (eg. by looking on the graduation B of the syringe).
$$X = \text{final volume passed for a } 10 \text{ mL instruction} = A - B$$
3. If the pump calibration is correct, X should be equal to Y = 10 mL.
4. If it is not correct, see next step.

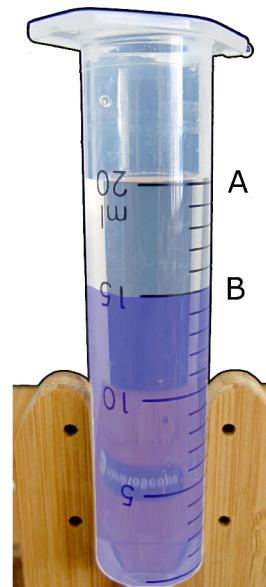


Figure 1 : Volume pumped. For example, here: $X = A - B = 20 - 15 = 5\text{mL}$. There is then an error in the calibration and we need to update the pump step according to this error.

8.2 Update pump step

1. Go to the hardware setting (fig.2).
2. Note the initial calibration "pump step per ml" here :
3. Calculate the "calibrated" pump step per ml such as **New Step = Y / X * old step**
4. Replace the "pump step per ml" parameter with this value.

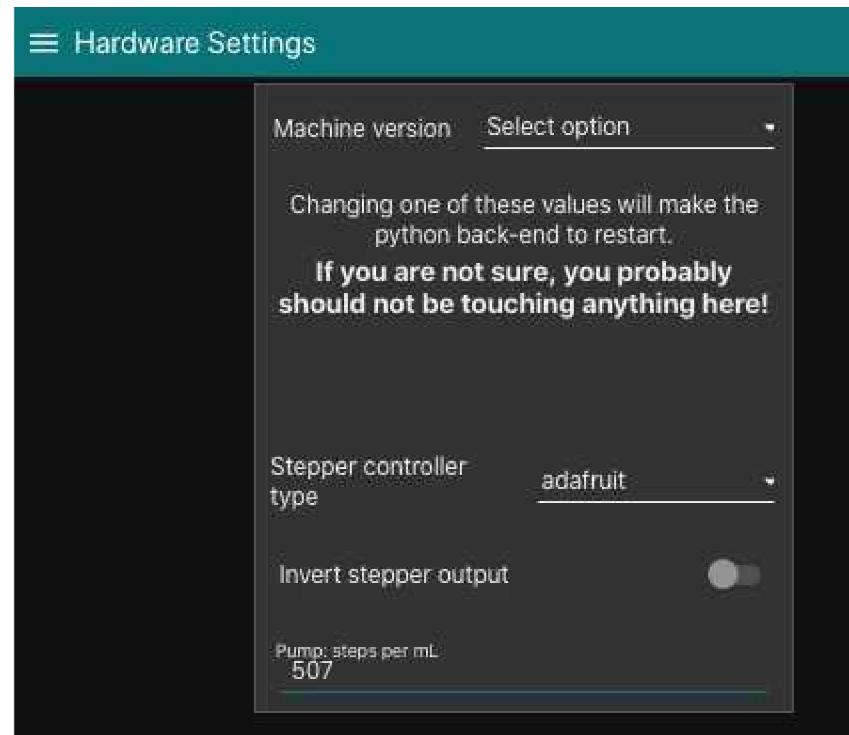


Figure 2: Hardware settings page (pump per step is at the bottom). This is only an example.

Size calibration

9 Size calibration

Safety information

Size calibration is an important process to get good data and should be absolutely done and noted. It will allow to know the real size of the plankton.

9.1 Set up the scale

1. Tilt the planktoscope on the side (camera on the bottom)
2. Remove the flowcell and place a micro metric ruler (or a millimetric one) on the sample stage such that the ruler is either vertical or horizontal but not in diagonal (fig.1).
3. Make the focus on the scale (fig.2).



Figure 1: PlanktoScope on the side with the micrometric ruler.

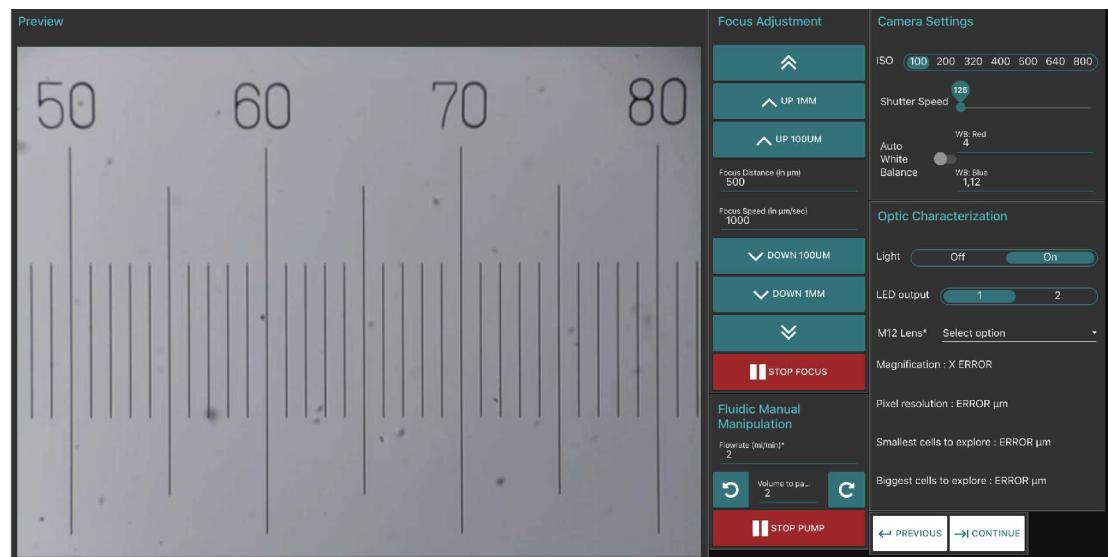
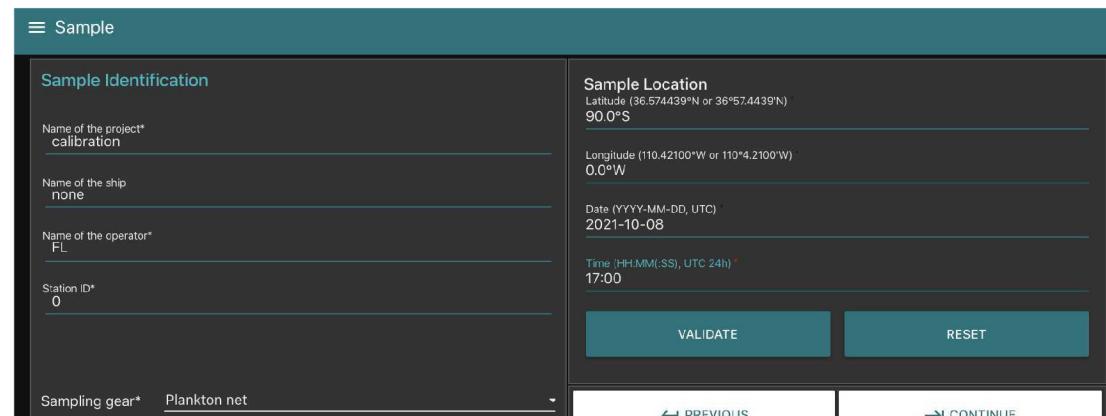


Figure 2: Focusing on the scale.

9.2 Take images of the scale

1. Fill metadata in the test mode in the sample tab (fig.3).
2. Take a few images, go on "acquisition" and put 1 or 2 images (fig.4). You should not use only acquired images to measure the real size of the pixels, and not the preview image as it will give you wrong results.



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

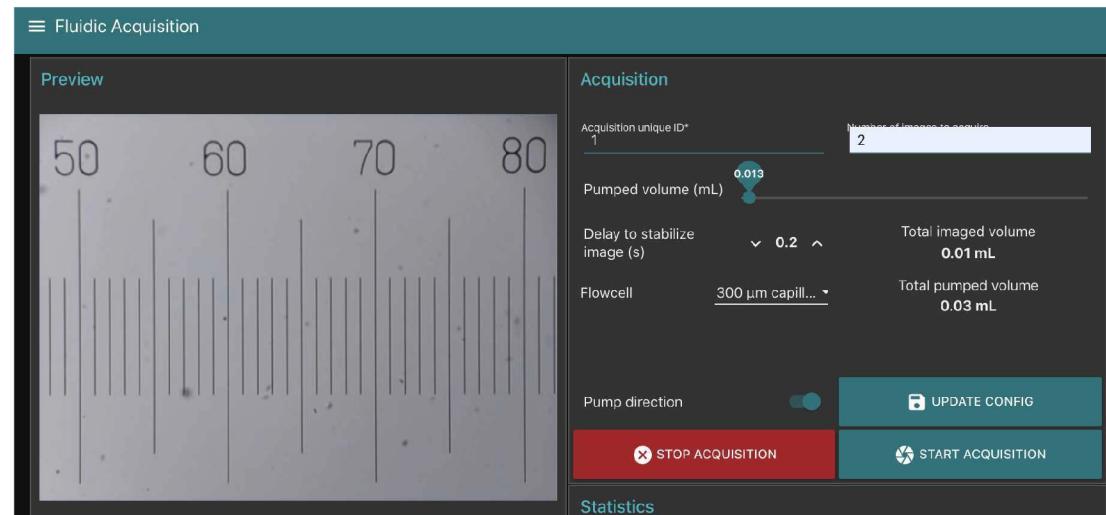
Date (YYYY-MM-DD, UTC) 2021-10-08

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

VALIDATE RESET

PREVIOUS CONTINUE

Figure 3: Example of metadata entered in the SAMPLE tab.



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 (right)

STOP ACQUISITION START ACQUISITION UPDATE CONFIG

Statistics

Figure 4: Here two images are acquired (and random information entered on the pumping one).

9.3 Measure the number of pixels

1. Download the images on a computer using filezilla, the datafile manager or the gallery according to your software version.

2. Open ImageJ.
3. File > Open. Choose the image.
4. Click on the line button (see fig.5) and trace a line.
5. Analyze > Measure.
6. Check the length value (in pixel).

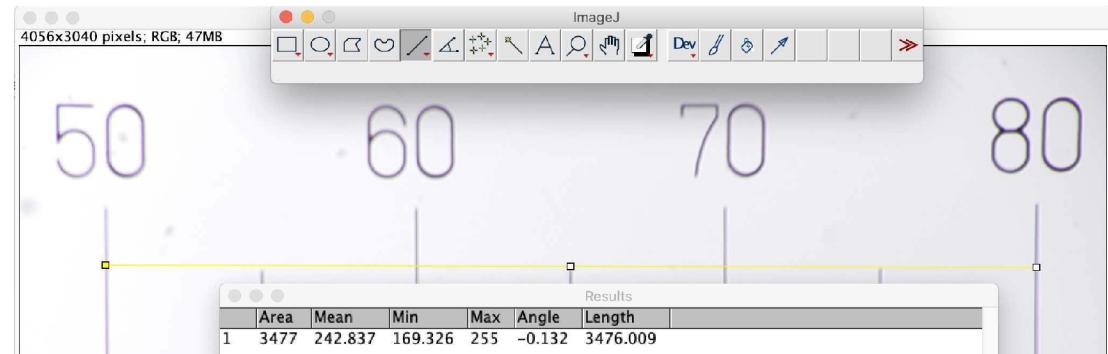


Figure 5: Using imageJ open your image (*File > open*), draw a line as long as possible (button in grey, click and maintain to draw the line) and measure it (*analyse > measure*). The line is 3476 pixel length for 3mm (i.e. one pixel is 0.86 with this example, which differ from the expected value of 0.75 for this camera).

9.4 Calculate the micron/pixel ratio

Calculate how much microns are represented by each pixel (The expected value for the default camera is expected to be around 0.75). You will need to do **Length(micron)/Length(pixels)**.

9.5 Update the micron/pixel ratio

Enter the calibrated pixel size value in the hardware setting (fig.6).

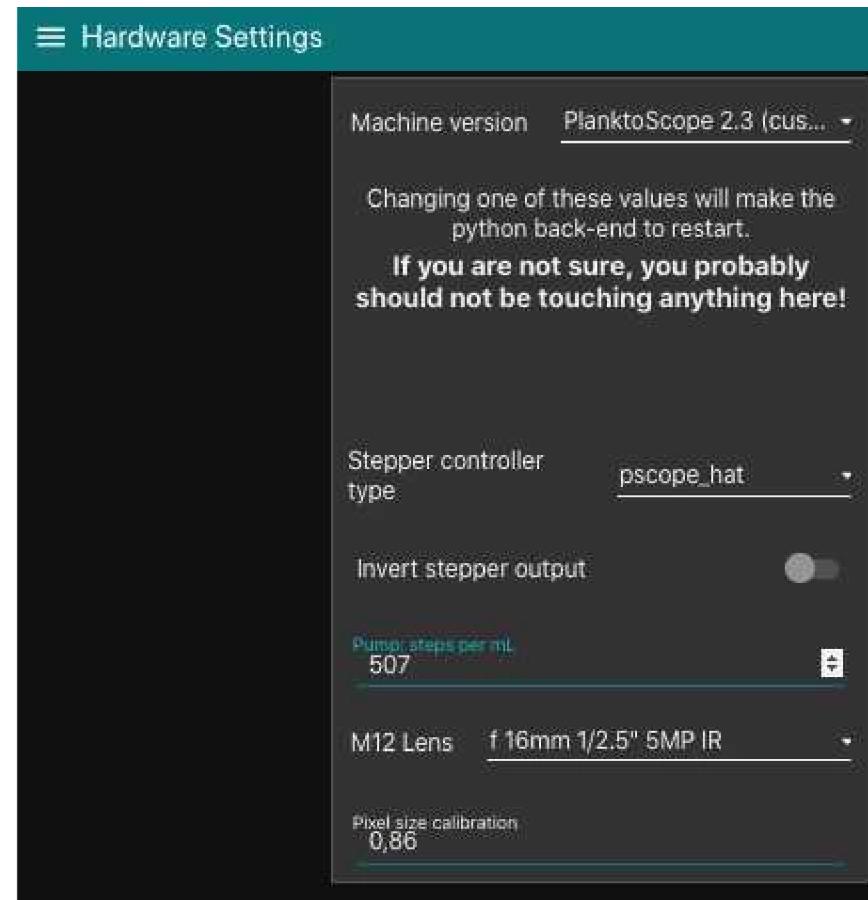


Figure 6: Pixel size calibration settings. This is only an example.

Get your sample

10 This protocol considers that a plankton net is used to collect the sample.

In this section, you will:

1. Collect your sample
2. Fill a log sheet
3. Filter your sample with a 200 μ m sieve

10.1 Collect your sample and the metadata

Safety information

Using logsheets:

- 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 information to get to quantitative sampling (see step 5.4)

You can find here a logsheet example (based on the datas needed in the SAMPLE Tab).

Operator:

Station ID:

Sampling gear:

Process time:

Net throw Lat:

Net throw Lon:

Net throw date/time:

(If it a horizontal sampling)

Net retrieval Lat:

Net retrieval Lon:

Net retrieval date/time:

Minimal fraction (μm):

Maximal fraction (μm):

Min depth (m):

Max depth (m):

! Critical information

Filtered Volume (L):

Concentrated sample volume (mL):

Dilution factor: (<1 if there has been a dilution)

Net opening dimension (mm):

Speed Through Water (kts):

10.2 Filter your sample

Safety information

Larger organisms may clog the flowcell. It is then necessary to filter the volume through a 200 μm sieve. Rinse the sieve using seawater and a squeezing bottle (helps to pass small objects).



Figure 1: How to filter the sample with the 200 μm sieve.

Pass the sample on PlanktoScope

11 Assemble and start the planktoscope before (see)

In this section you will:

1. Fill the metadata of the logsheet in the sample tab
2. Check the focus and the flowcell (image quality)

3. Put the light on and the bubbler
4. Put your sample and check the concentration of your sample
5. Dilute your sample if necessary
6. Pass sedimented organisms
7. Launch an acquisition

11.1 Fill the metadata

Go to the "sample" page and fill the metadata. This step is critical because those data are the ones that will make your sample usable or not.

Safety information

If the PlanktoScope has already been used before, the old metadata are kept. It can be useful, but do not forget to change them if it is necessary.

- **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

Figure 1: Sample ID parameters.

- 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 (to remove too large organisms)
- **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.

Safety information

Known bug: If filtered volume is provided but also initial/final size, calculation from this latter may replace the measured filtered volume.

In all cases the **diameter of the net opening** will be needed to calculate the filtered volume (only if you used a plankton net).

- Note the **mesh size** used for collection in "minimal fraction size" (it will be used afterwards in the segmentation process, object smaller than this won't be segmented);
- The "**Maximal fraction size**" is the mesh size used to filter the sample during preparation (It must have been done at 200µm so as not to block the fluidic circuit);
- The "**Filtered volume**" is the volume passed through the net during sampling. It is better if you recorded it but could be calculated from other parameters. So, 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; and in all cases the diameter of the net opening (to be able to calculate the volume afterwards).
- "**Concentrated sample volume**" is the volume recovered by the net.
- If a **dilution** has been done, note the "dilution factor" (if not, write "1"). <1 if it is diluted, >1 if it has been concentrated.

Sampling gear*	Plankton net
Minimal fraction size (µm)	20
Maximal fraction size (µm)	200
Min sampling depth (m)	0
Max sampling depth (m)	2
Filtered volume (in L)	
Concentrated sample volume (mL)	138
Dilution Factor	
Speed Through Water	
Net opening dimension (mm)	300

Figure 2: Sampling parameters. This is only an example.

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

Note

When you click on "Validate", the coordinates will disappear in any case. You can check that they are correctly registered in the "acquisition" tab. If not, you will have a warning.

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

Figure 3: Date and time of the sample. This is only an example.

11.2 Check flow cell alignment

1. Turn the light on.

2. Check for lens alignment with the flow cell. Move slowly the flow cell receptacle until there is no black background like in the image below.

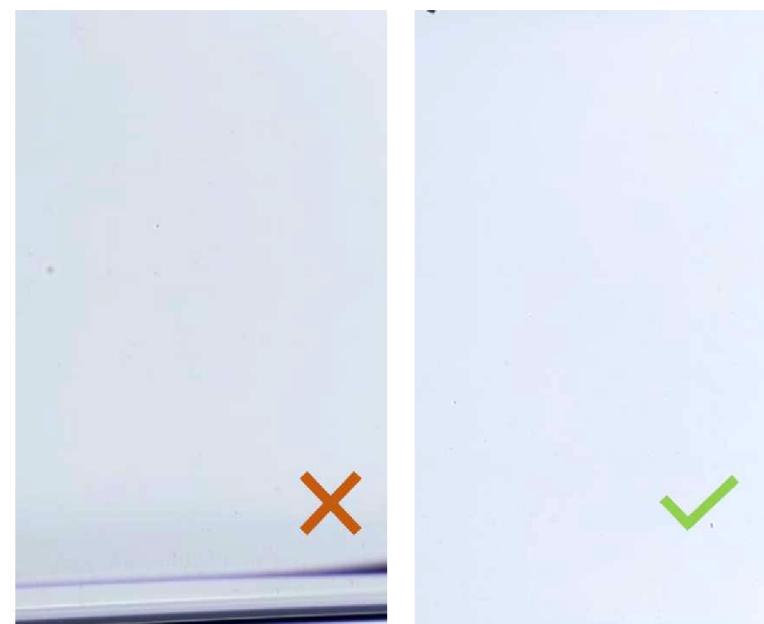


Figure 4: The figure on the left shows a misaligned flowcell. The background should be homogeneous like in the figure on the right.

11.3 Do the focus

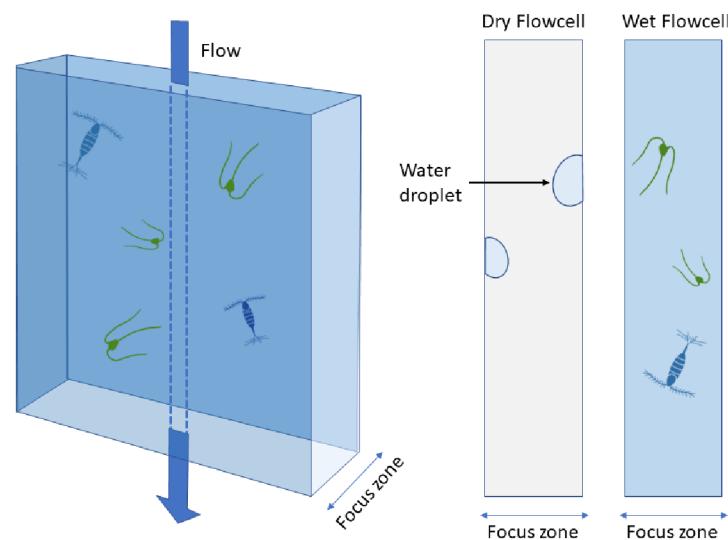


Figure 5: Focus zone of the flowcell.

Go to the OPTIC CONFIGURATION tab:

1. Turn the light on if not already done.
2. Check focus on the two sides of the flow cell, and try to have a focus between the two sides.
In the figure above, you can see that on a dry flow cell that has been exposed to water, there are still water droplets on both sides. Use them as an indicator to do the focus.
3. You can also do the focus on one side, and then move the focus by the size of the flowcell divided by two. For a flowcell of 300 microns, you should move the focus by 150 microns in the opposite direction.

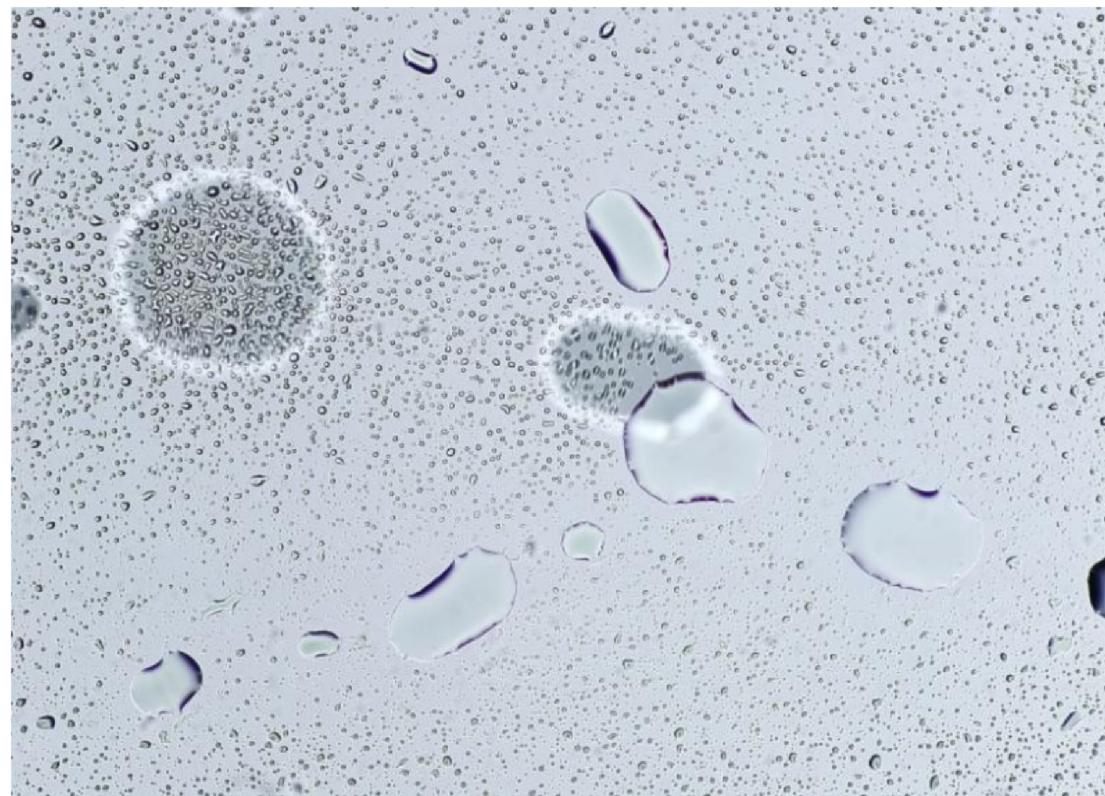


Figure 6: Water droplets. Here, there is a good focus on the front face, and you can see two other water droplets badly focused on the back side of the flow cell.

Note

tip#1: start using the "1mm" buttons, then the 100 μ m buttons and finish by typing 25 or 50 μ m adjustments in the middle box and pressing external arrows of focus;
tip#2: you can connect your phone or a tablet to the planktoscope to have controls on the focus while checking a zoomed portion on the streamed image on another device.

11.4 Put your sample in the PlanktoScope

1. Close the stopper
2. Fill the sample in the syringe. For this you can just remove the full sample holder (and fill it on top of a sink (to not risk spills on-top of the planktoscope).
3. Reconnect the syringe to the pump
4. Open the stopper
5. Place the bubbler and adjust the flow to 1 bubble/second.

Safety information

Never forget to open the stopper. If the stopper is closed for a long time, it can deteriorate the fluidic path.

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. **You should agitate your sample using bubbling and use pumping rate enough to avoid sinking/clogging of sample.**

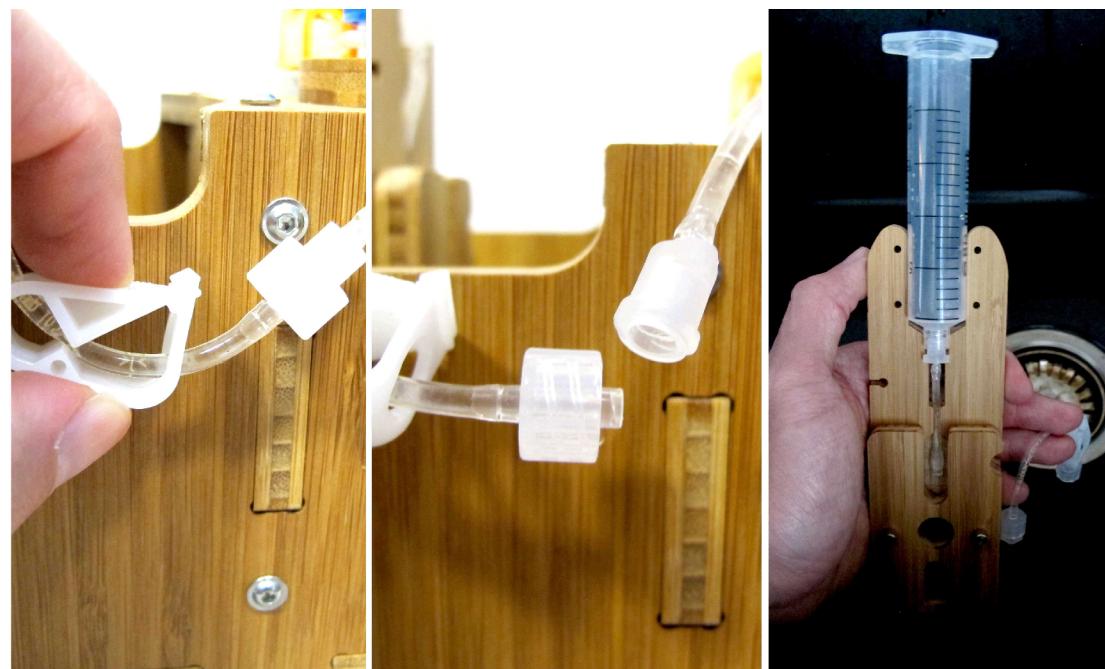


Figure 7: How to remove the fluidic part containing the flowcell to fill the syringe with water.

11.5 Dilute your sample if necessary

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 in metadata !**

Safety information

Having too many objects per frame will :

1. increase the probability to aggregate objects (making them impossible to count or identify)
2. increase the probability of clogging the fluidic system
3. create artefacts during the segmentation step

11.6 Pump sedimented particles

In the OPTIC CONFIGURATION tab, pump with high flow rate a good amount of water (goal: remove plankton that have sunk in the fluidic system). **You do not need to pump a large amount of your sample, 1 mL is sufficient.**



Figure 8: How to pump a specific volume without image acquisition.

*Be careful, even if the volume chosen here will not impact the future acquisition (it is completely independent), the flowrate will be the one used during the acquisition. It is recommended to have a low flowrate for an acquisition (around 2 ml/min). **Do not change the flowrate during an acquisition and stay around 2 ml/min after you pumped sedimented organisms.***

Safety information



Figure 9: How to unclog the fluidic path. *If your fluidic system is not optimized to avoid plankton sedimentation, some plankton could accumulate in the fluidic system. This can be checked by pinching the tube halfway in between the flowcell and the pump during 1-2 seconds (to accumulate suction pressure) and releasing it. If a large quantity of plankton passes suddenly this means that plankton have sedimented between the syringe and the flowcell.*

11.7 Acquisition

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 :

- Avoid plankton sedimentation in the fluidic system
- 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 or more (e.g. if you have 10 objects per image, imaging 100-200 frames would be enough). Lower numbers of objects would be statistically problematic.

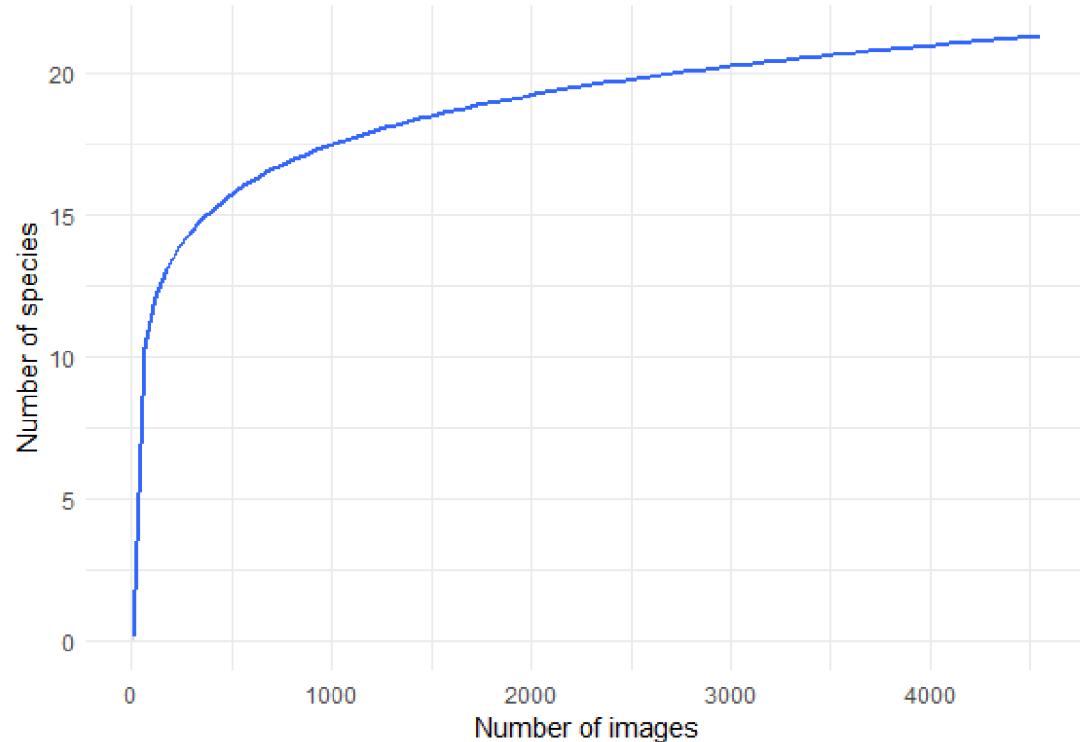


Figure 10: Number of species per number of images acquired for a random sample. Note that the number of species starts to stabilize around 2000 images here.

Acquisition

Acquisition unique ID*	1	Number of images to acquire	100
Pumped volume (mL)	0.03	Total imaged volume	0.21 mL
Delay to stabilize image (s)	0.5	Total pumped volume	3.00 mL
Flowcell	300 µm capill...		
Pump direction	<input checked="" type="checkbox"/>	<input type="button" value="UPDATE CONFIG"/> <input type="button" value="STOP ACQUISITION"/> <input type="button" value="START ACQUISITION"/>	

Figure 11: Acquisition parameters. **This figure is not a recommendation**, as it depends on the number of objects you want to acquire and the type of flowcell you use.

- **Volume to pump** is the volume pumped in between two images: it should be large enough to avoid taking twice the same object in photo; avoid large sedimentation in the fluidic system; avoid objects to stick on the flowcell. It is recommended testing it in order that the volume passed between two images correspond at least to 5-10 times to the volume imaged (see here the discrepancy between imaged volume/pumped volume).

- **Delay to stabilise image** is the time lag in between the stop of the pump and the acquisition of the image. **It should be large enough to avoid objects moving while imaged.**

1. Go to fluidic acquisition and start the acquisition.
2. First take two or three images to check if the focus is good. If it is not, try to do it again directly on the plankton.
3. Change the acquisition ID (the old one is used by the focus test).
4. Launch the real acquisition.
5. Wait for the acquisition to be done.
6. Results can be consulted by consulting the gallery or the file manager (*data > export*).

Safety information

The Planktoscope is using a "rolling shutter camera" which means that there is a small delay in between the first line of pixel imaged and the last line of pixel imaged. To overcome this, it uses a "stop and go" strategy where the imaging only takes place when the flow of the pump is stopped. Not setting this properly will generate artefacts, swimming organisms will also suffer from this (example below).



Figure 12: Copepod nauplii moving while imaged.

Segment the acquisition

12 Segmentation

1. Go on segmentation and click on the "update acquisition's folder list".

2. Select the samples you wish to segment. Note that you can select either the parent folder or select the file itself.
3. Setup the different options of the segmenter.

Safety information

Even if the segmentation process can take a lot of time, it is not recommended to start the acquisition of a new sample during this part in case the results are corrupted. You should do the cleaning of the fluidic path and process a new sample only if you are sure that all your images are not corrupted.

- Recursive folder means that it will segment all samples within a selected sample
- Ecotaxa archive: it will create a zip file containing all files needed for a easy importation within ecotaxa
- Force rework: if yes it will re-segment samples already segmented
- Keep objects: it will keep the final segmented images visible in the planktoscope (that could be accessed by the gallery in the objects folder)

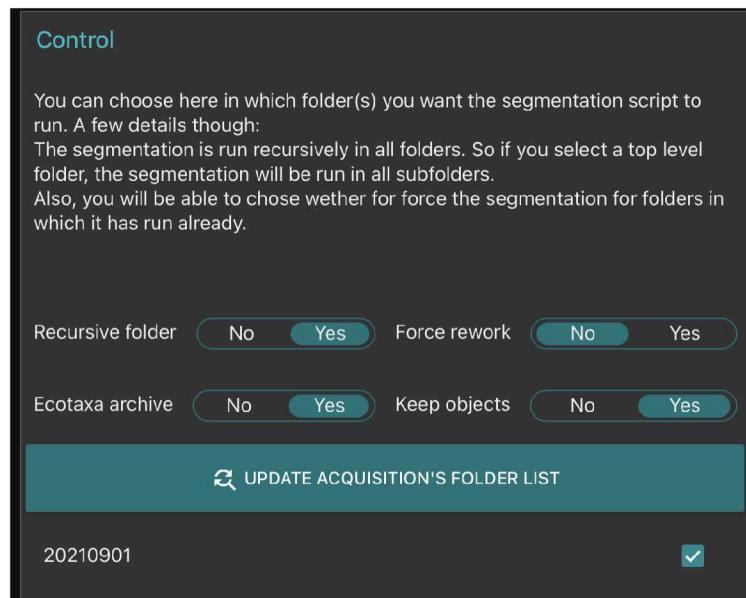


Figure 1: Where to find the folders containing the images to do the segmentation. **Do not forget to update the folder list.**

1. scroll down and click on start segmentation
2. Wait for the segmenter status to turn to "done"

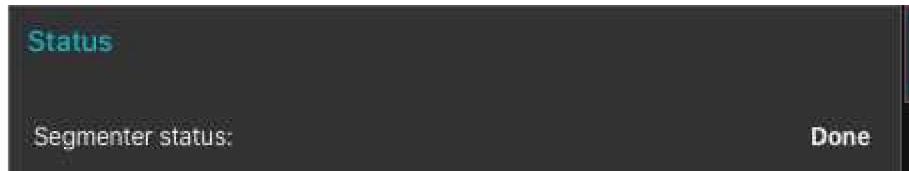


Figure 2: End of the segmentation.

Export your data on your computer

[go to step #6 How to export data](#)

Clean the planktoscope

13

Cleaning

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 #11.6](#))
7. Drain again the syringe (**repeat steps 2-7 at least 2 more times** until no plankton is visible on the camera)
8. Finally drain the system

If not used immediately

1. Put 20 mL diluted bleach
2. Leave 15'
3. Drain the content (high pump speed)
4. Put 10 mL fresh water
5. Drain the content (high pump speed)
6. If there are traces of calcification, use a diluted acid solution (like HCl diluted by 6).

Upload your images on EcoTaxa

14

In this section you will learn to :

1. Do your first connexion on EcoTaxa
2. Create a new project

3. Connect to EcoTaxa with filezilla
4. Import data of the PlanktoScope to EcoTaxa

First connexion

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

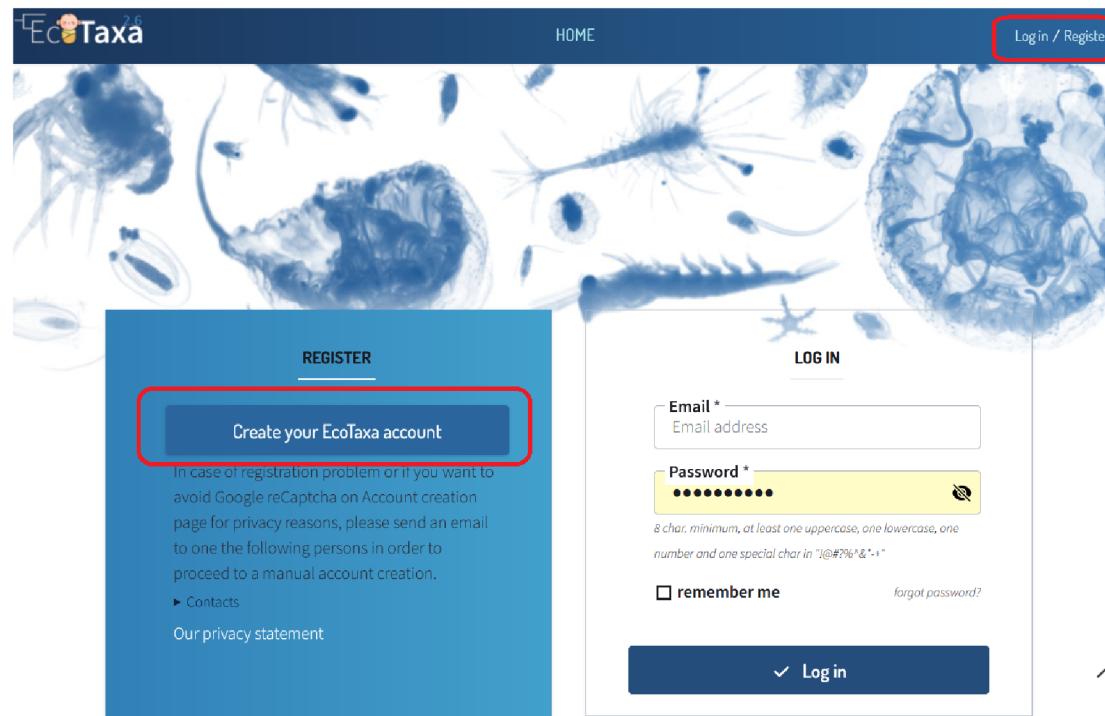


Figure 1: Log in/Register interface.

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



Figure 2: Ecotaxa account creation interface.

14.1 Projects

Once logged in, 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 on "**contribute to a project**" on the main page.



Figure 3: Accessible buttons on the main page.

14.2 Create a project

Go to "*Contribute to a project > Create a new project*". You can create your own project on which you will be able to import, visualise and classify images.

In the creation panel, you can (fig.3):

- Add the title of your project
- Describe your project
- Comment your project

- Define the instrument used (here, the PlanktoScope)
- Choose if you want to annotate (define taxonomy) or only explore images, etc. and who can see your project
- Define what pre-trained Deep Learning features to use on your project (it is recommended to use «Planktoscope_2022-09 » unless you see a more recently trained model on planktoscope image)
- Choose a license for your images
(it is recommended to use one of the CC-BY one or CC-0 if you want data to have a future use for science)
- Define a list of taxa to help you classify your sample (in the "Taxonomy" field)
- 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 :

area=area

meanhue=meanhue

meansaturation=meansaturation

meanvalue=meanvalue
- Invite new contributors/viewer/manager

If you want to edit the settings later, go to "edit project settings" (fig.4).

The screenshot shows the 'NEW PROJECT' interface. At the top left is a button labeled 'PICK FROM PROJECTS'. The main area contains several input fields and dropdown menus:

- Title ***: A text input field containing 'project title'.
- Instrument ***: A dropdown menu.
- Description ?**: A text area containing 'project description'.
- Status ***: A dropdown menu with three options: 'Annotate' (selected), 'Explore Only', and 'Annotate No Prediction'.
- Visible for all visitors (only validated objects)** : A checkbox.
- Comments ?**: A text area containing 'project comments'.
- Deep feature extractor ?**: A dropdown menu.
- License ? ***: A dropdown menu with five options: 'CC0 1.0', 'CC BY 4.0', 'CC BY-NC 4.0', 'Copyright', and 'not chosen'.

Figure 3: New project interface.

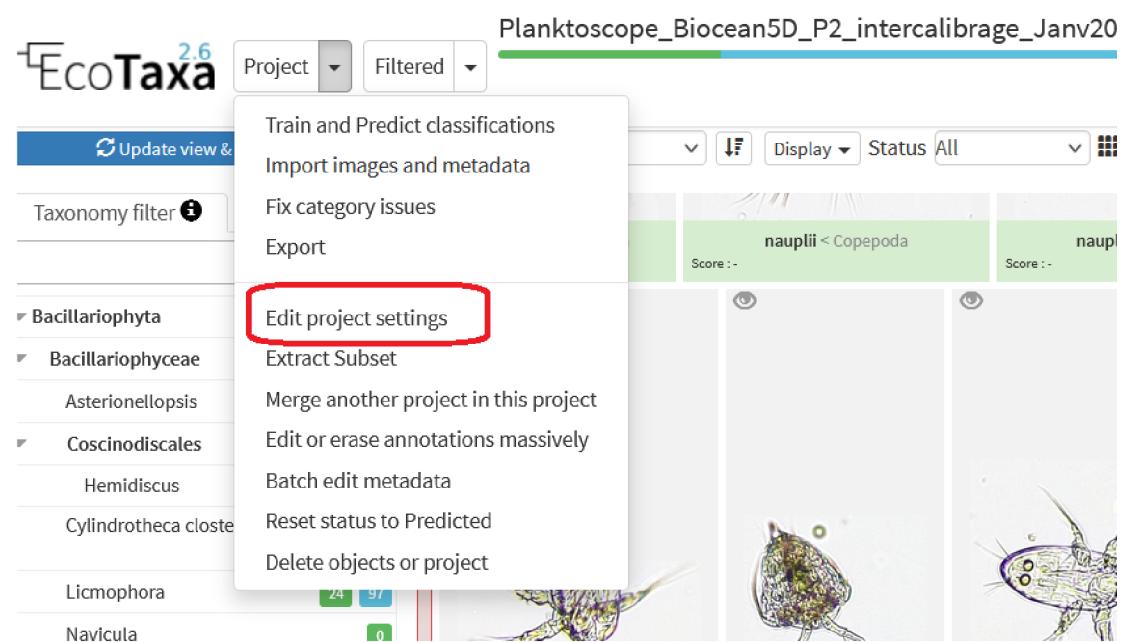


Figure 4: "Edit project settings" button.

14.3 Connect to EcoTaxa ftp

Safety information

In v.2023, you can also download the zip file directly from your computer, without using FileZilla. Otherwise, FileZilla is recommended for huge amounts of data.

1. Upload the ecotaxa archives (see step 6-7) on the EcoTaxa ftp
2. 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/PLANKTOSCOPE).

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).

14.4 Import data in your project

1. In your project/ on your project options button, select import images and metadata (fig.6).
2. Locate your file on the ecotaxa ftp folders and import it (only works for one zip file at a time for now; fig.7).
3. Check the quality of your images and the quality of the segmentation once the images are imported.

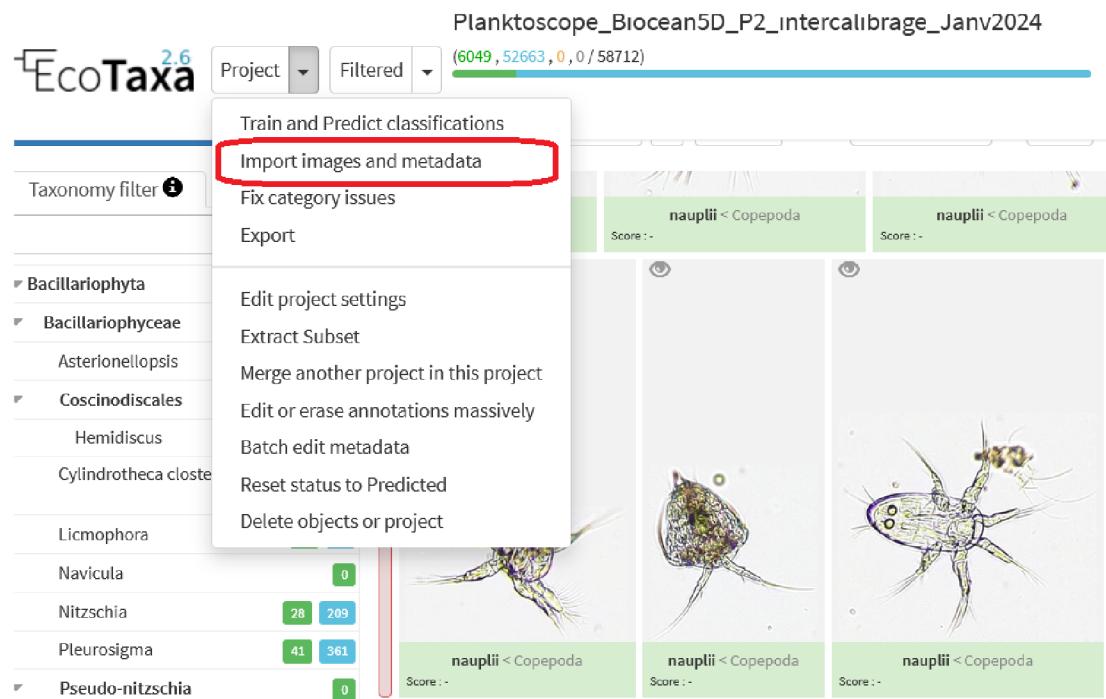


Figure 5: How to import images and metadata.

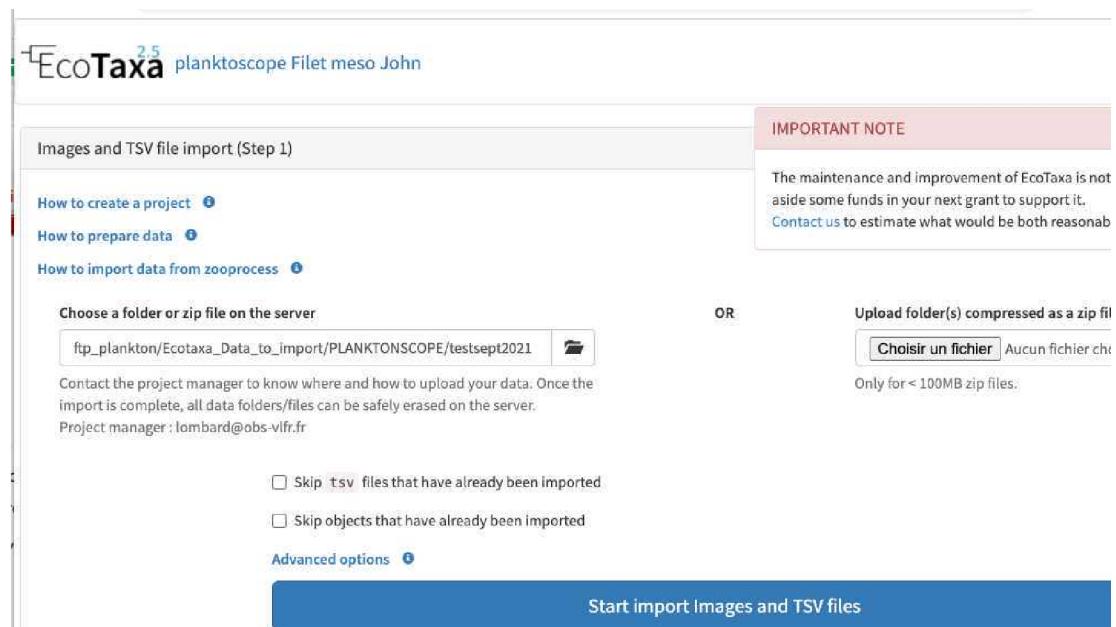


Figure 6: Data import interface.

How to use efficiently EcoTaxa

15

In this section, you will learn to:

1. Use filters
2. Validate taxonomy
3. Do a prediction
4. Export the results

Note

For more details, please check the online manuals <https://sites.google.com/view/piqv/piqv-manuals/ecotaxaecopart-manuals?authuser=0>

15.1 Use filters wisely

There are three layers of filters in EcoTaxa:

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

2. Filters on sample parameters (date, location, etc).
3. The quick access filters (top bar). Quick filters are pretty useful since you can sort objects by specific values (eg. mean saturation in fig.3, to quickly observe objects that have lots of chlorophyll). You can revert the sorting order of those filters by ascending or descending order.

Filters are additive (fig.2), 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).

A Taxonomy filter A Other filters

Bacillariophyta	0		
Bacillariophyceae	84	2300	
Asterionellopsis	308	619	
Coscinodiscales	96	295	
Hemidiscus	1	1	
Cylindrotheca closterium < Cylindrotheca	4	482	
Licmophora	24	97	
Navicula	0		
Nitzschia	28	209	
Pleurosigma	41	361	

B Taxonomy filter B Other filters

Share page X Clear all filters Advanced Clear

Sample

Depth Min [m] Max [m] Clear

Location

West North East

South

Open map

Update view & apply filter Save filters Apply saved filters and update view

C

Select all Up Score Display Status All 100 Q% 40 Search Comment Print Refresh

Figure 1: (A) Taxonomy filters; (B) Sample filters; (C) Status and feature filters.

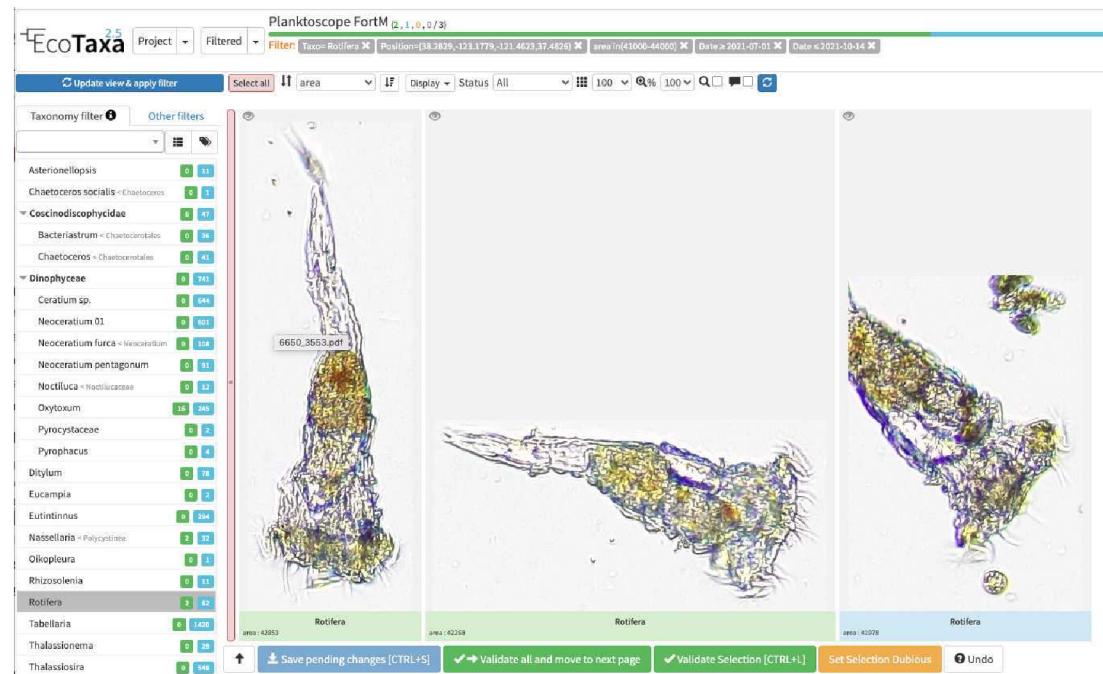


Figure 2: Multiple filters applied in a project. At the top of the figure you can see that the filters allows to see only the taxon "rotifera" of a specific size (area of the image), at a specific time and location.

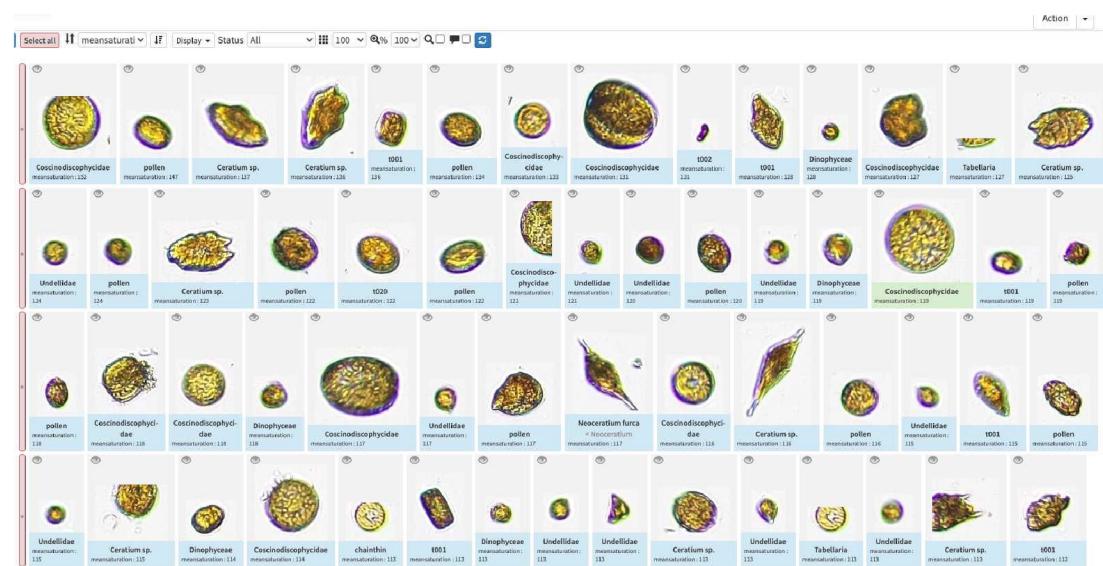


Figure 3: Objects sorted by the mean saturation.

15.2

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

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

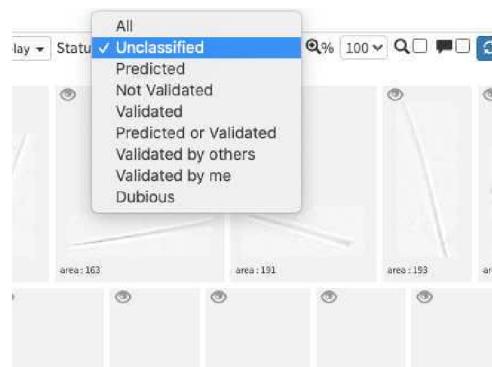


Figure 4: Unclassified images.

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).



Figure 5: Types of images status. In blue predicted ones, green validated, orange classified as dubious.

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.

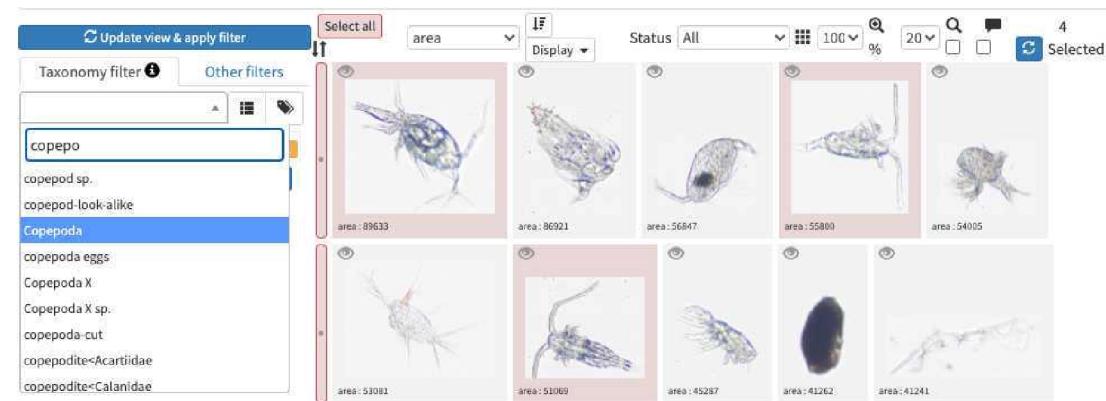


Figure 6: Typing "copepo" brings several results.

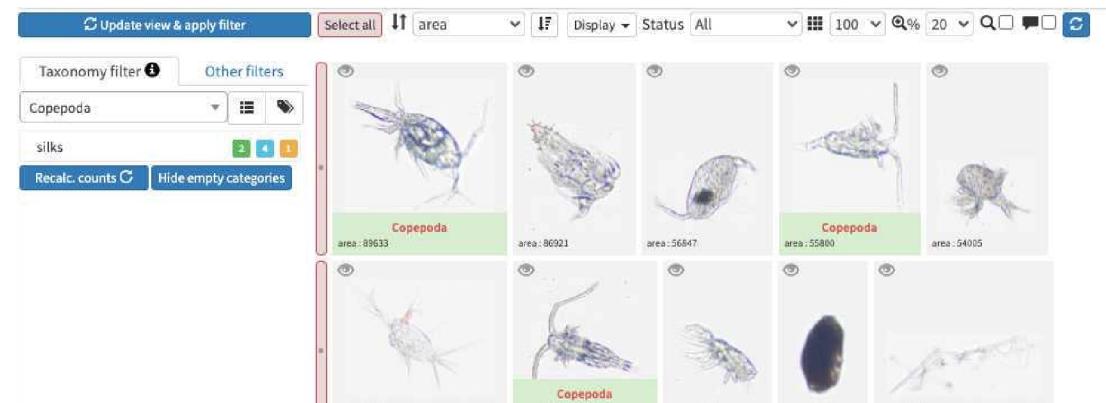


Figure 7: Once validated the name appears in red below the images.

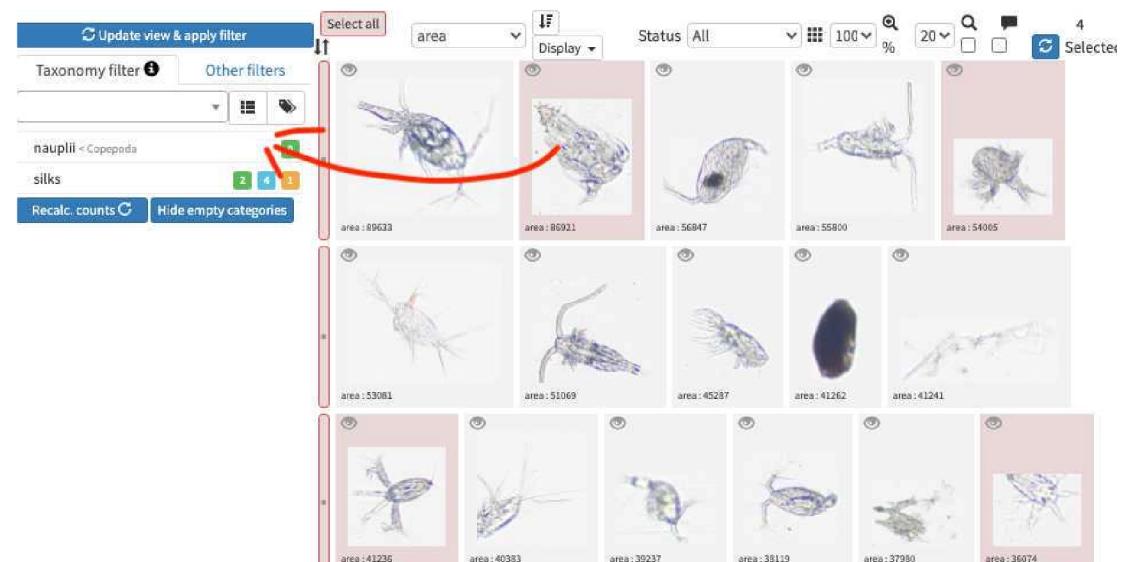


Figure 8: Sliding into existing categories also works.



Figure 9: Dont 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!

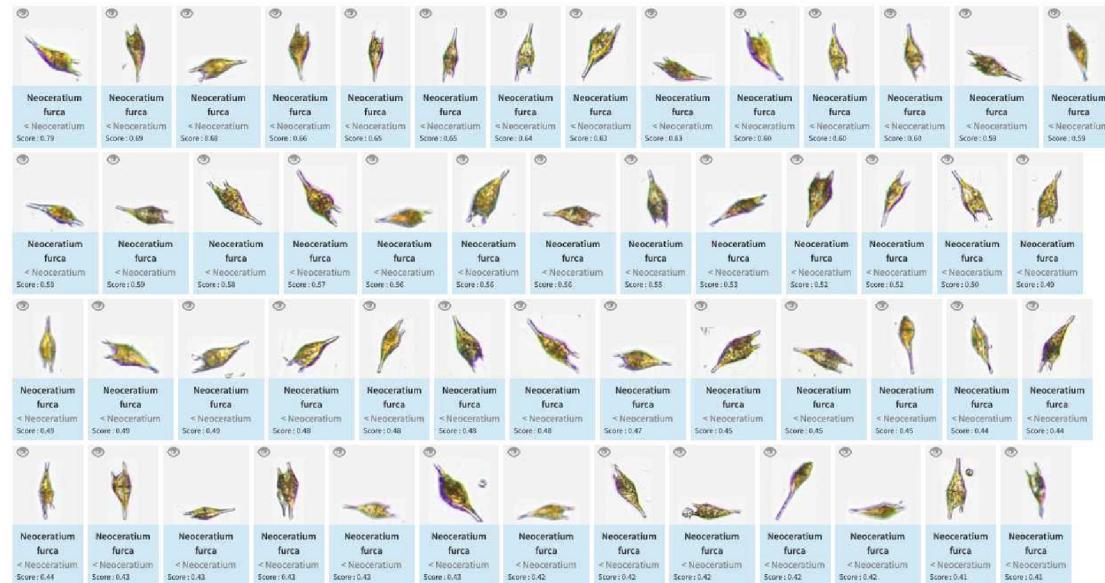


Figure 10: Example of (well) predicted objects which would be easily validated.

15.3 Prediction

Do not hesitate to "predict" your project right away (even with a project/instrument that has nothing to do, even if it is recommended).

Safety information

You can use the PlanktoScope machine learning algorithm in your project. *Go to Project > Edit project settings* and chose the SCN Network "planktoscope_2022-09". Don't forget to save changes.

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

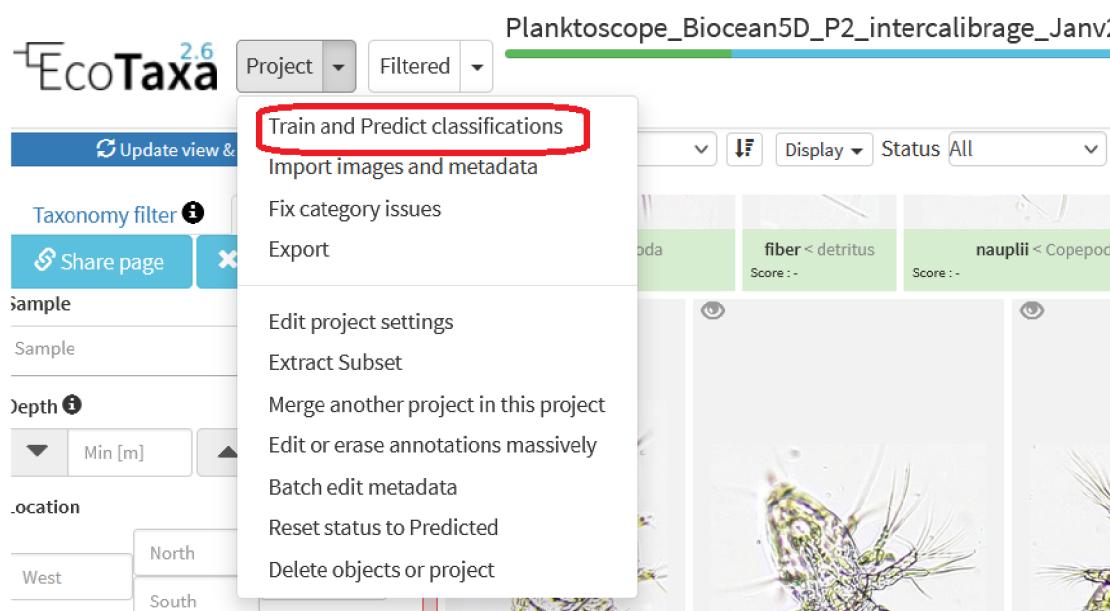


Figure 11: Click on the button "Train and Predict classifications" to start a prediction

- You can select any of the pre-existing projects (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.

Projects that could be used for first prediction :

- #6818 - MOOSE-GE-2022_tests_ID_vignettes (Med sea; Processed with current segmenter; Fully validated) <https://ecotaxa.obs-vlfr.fr/prj/6818>
- #9621 -Planktoscope APERO_PP_Phytonet_35mu <https://ecotaxa.obs-vlfr.fr/prj/9621>
- #9942 Planktoscope APERO_TH_Phytonet_35m (PlanktoScope; Fully validated) <https://ecotaxa.obs-vlfr.fr/prj/9942>
- #10 056 - AtlantECO_P2_Ada_2023 (PlanktoScope; 60% validated) <https://ecotaxa.obs-vlfr.fr/prj/10056>
- #6765 - Planktoscope Reference (Processed with other segmenter, **works only with adding Deep Learning features into play**); Fully validated) <https://ecotaxa.obs-vlfr.fr/prj/6765>

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 ≥ 10 Instrument: Filter

Project deep features model: flowcam.
 Model is: **empty** ⓘ

# - 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

Figure 12: Prediction learning sets.

- Push the button "Next: Choose objects in selected projects". You then have the possibility to select what types and quantity of objects to consider. It is recommended to try to avoid selecting too much objects in one category to partly correct the usual strong imbalance between categories (In the fig.13, it is limited to 500 objects per group).

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: **500** objects per category. ⓘ
 Total is currently 2877 objects.

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

Figure 13: Choice of objects to train the machine learning algorithm.

- Click on "Next: Choose features in selected objects". 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)
- Click on "Start prediction task"

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"/>	perimareaexc <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"/>						

Figure 14: Prediction features and settings.

- 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 determines 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).

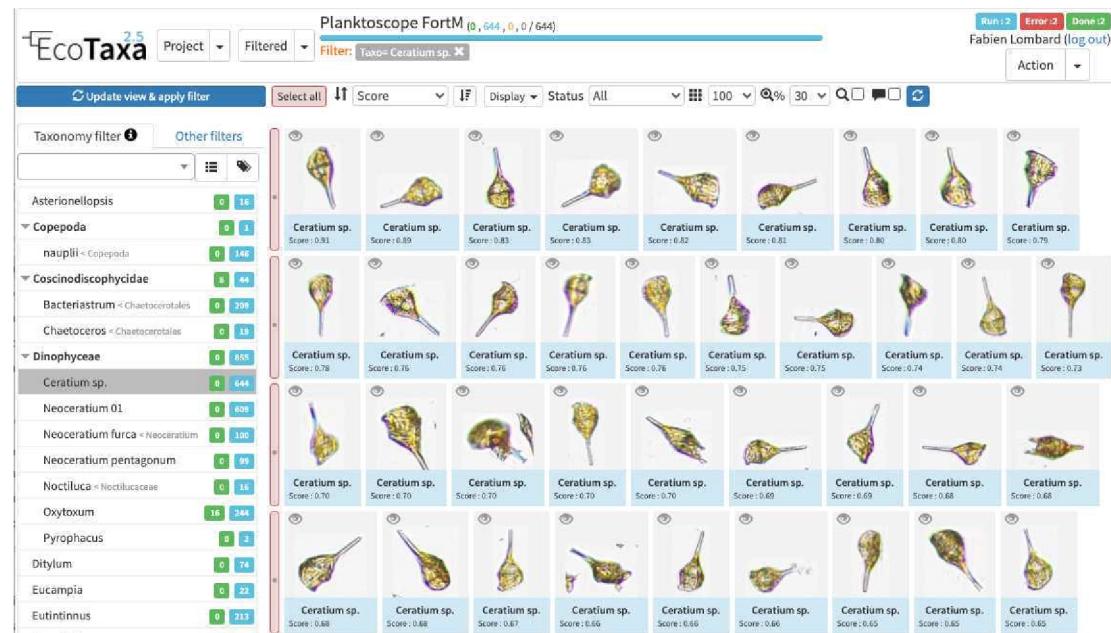


Figure 15: Example of images sorted by score of prediction.

Safety information

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 optimized 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).

15.4 Export your results from EcoTaxa

Once fully validated, export your results (lots of different solutions exist, the easiest to understand being the summary export with count per sample).

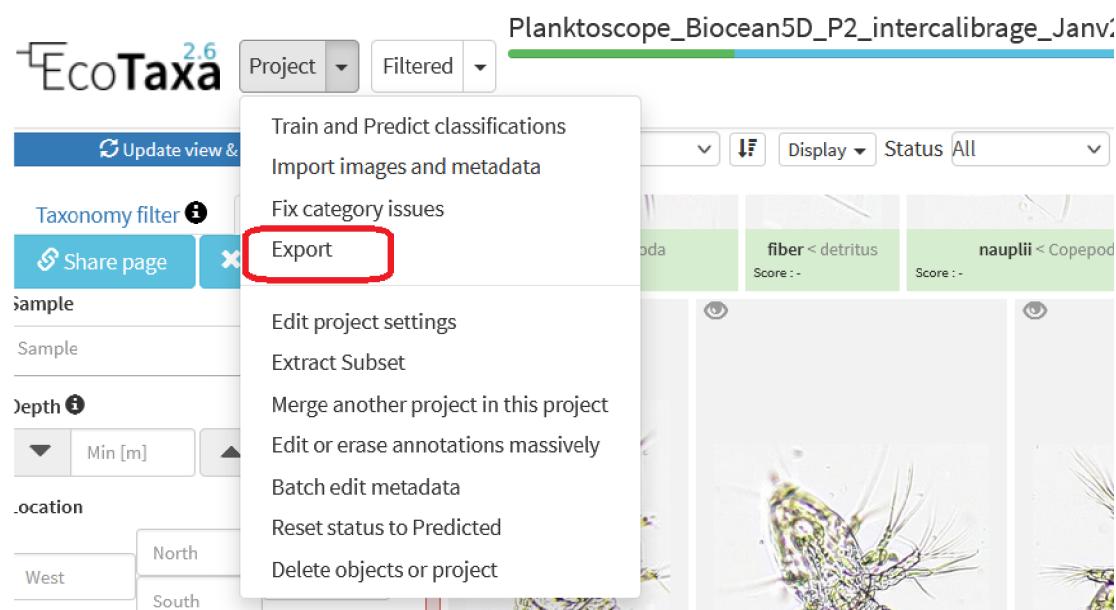


Figure 16: How to export data.

Full export Planktoscope_Biocean5D_P2_intercalibrage_Janv2024

Active Project Filters

[CUSTOM](#) [BACKUP](#)

- Object Data (median,mean,x,y,...)
- Process Data (software,version,...)
- Acquisition Data (Resolution,...)
- Sample Data (lat,long,date,...)
- Historical Data
- Comments
- Use coma as decimal separator
- Format dates and times using - and :
- Internal Ids (including taxonomic source Id)

Export images

NO Images

Split in multiple files by

NOT Active

[Start Task](#)[Cancel](#)**Figure 17:** Data export interface.

How to compute biovolumes

16 Calibrate your data

Ecotaxa generate a lot of interesting variables to analyse your sample. Only a few of them are depicted here, but don't hesitate to explore the other ones.

Before any analysis, it is important to relate all the parameters computed by Ecotaxa with the acquisition parameters. You will need :

Variable	Unité EcoTaxa	Unité Script	Calcul
Sample_concentrated_sample_volume ($V_{concentrated}$)	mL	m^3	$X/1\ 000\ 000$
Acq_imaged_volume (V_{imaged})	mL	m^3	$X/1\ 000\ 000$
Sample_total_volume (V_{total})	L	m^3	$X/1\ 000$ $(=1 \text{ si NA})$
Pixelsize	μm	mm	$X/1\ 000$
Major	px	mm	$X * \text{pixelsize}$
Minor	px	mm	$X * \text{pixelsize}$
Area_exc	px	mm^2	$X * \text{pixelsize}^2$
Area	px	mm^2	$X * \text{pixelsize}^2$

Table 1 : Transformations to apply to essential variables.

It will allow you to compute the conversion factor "conver". The dilution is a factor that should be < 1 if a dilution has been operated and > 1 if the sample has been concentrated (it is not the sample concentrated volume, which is the original volume taken from the collector) :

$$conver = \frac{V_{concentrated}}{V_{imaged} * V_{total} * Dilution}$$

Safety information

Do not forget to convert all the parameters like above. Otherwise, it will produce wrong results.

Compute Biovolume

There are three methods to calculate the biovolume (BV) of an object.

Ellipsoid:

$$AR = \pi * \frac{major}{2} * \frac{minor}{2}$$

$$BV = \frac{4}{3} * \frac{minor}{2} * AR$$

Plain:

$$ESD = 2 * \sqrt{\frac{area}{\pi}}$$

$$R3 = \left(\frac{ESD}{2}\right)^3$$

$$BV = \frac{4}{3} * \pi * R3$$

Riddled:

$$ESD = 2 * \sqrt{\frac{area_exc}{\pi}}$$

$$R3 = \left(\frac{ESD}{2}\right)^3$$

$$BV = \frac{4}{3} * \pi * R3$$

Maintenance of your PlanktoScope

17 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 it occurs during sample acquisition, even abort this latter, take care of the clog, maybe dilute the sample and restart acquisition while noting that the sample got diluted in the metadata).
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.
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.
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 crystals 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.

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 tissues are a 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 require 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 ! NEVER touch it, only use dry gas.

Regularly calibrate the pump and the WB.

Update the software or reset the PlanktoScope

18

If you need to update the software, or if there are a lot of bugs and you want to reset it, follow this procedure.

1. Withdraw the micro SD card at the bottom of the PlanktoScope with a fine forceps (see fig.1). The fine forceps is not provided.
2. Connect the SD card to your computer with a SD card adapter if needed (you can find one in the supplementary materials box).



Figure 1: How to withdraw the micro SD card.

1. Open BalenaEtcher > Flash from file

2. Choose the desired version of the software (.img file). You can find the actual one on the github of the PlanktoScope in the "external links" section of the protocol.
3. Choose the SD card you want to flash.
4. Click on "flash" and wait until it is flashed.
5. Eject it from your computer and put it back in the PlanktoScope with a fine forceps.

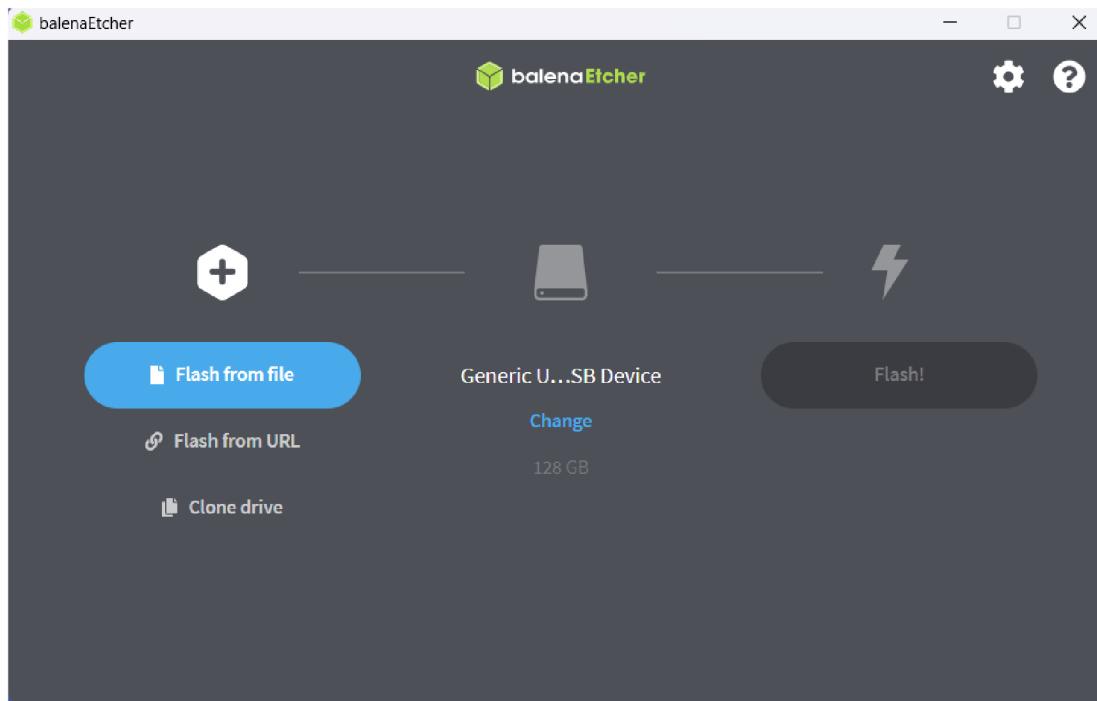


Figure 2: BalenaEtcher interface.

Troubleshooting

19 The flow cell is clogged with plankton

Why this happens:

- First this may happen if your sample does not have been pre-filtered (it is recommended to do a pre-filtration to 200µm).
- It may also happen if your sample is too concentrated. If you got more than 20 plankton objects per image this may already be the case, dilute your sample and fill the dilution factor in the sample metadata.
- If you forget to agitate your sample using a bubble or if you let your sample to stagnate for too long in the fluidic system.

Unclogging the flowcell:

- Try to pinch the tube in between the flowcell and the pump (while the pump is running).

- Try to do the same while pumping in the reverse direction (eventually at high speed- see pump controls in optical configuration page).
- Dismount the flowcell but keeping the luer lock connectors on it. on the side which was connected with the pump, either blow in it or connect a syringe and pass air/water to chase the blocked plankton.

19.1 The image is partly blurred

Why this happens:

- The focus is correctly done, but the flowcell is not well positioned.

How to correct it:

- Try to adjust the position of the flowcell with tape.

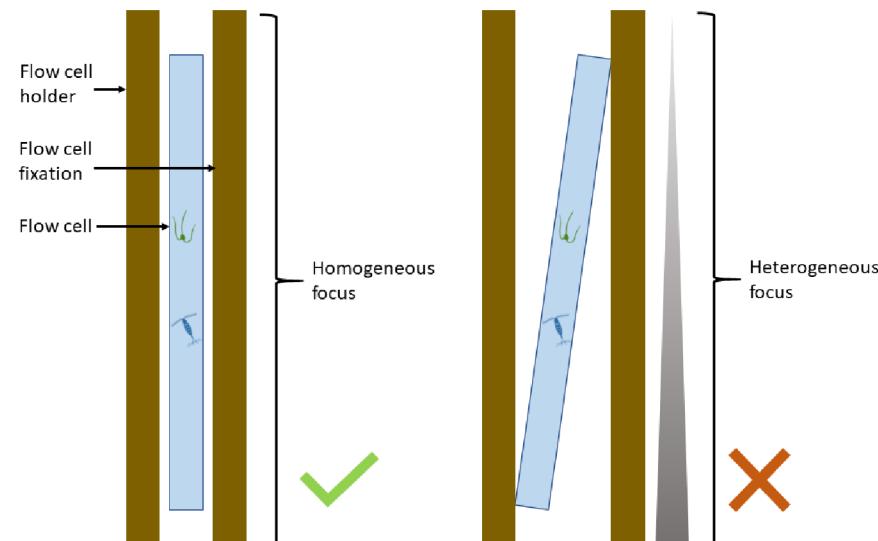


Figure 1: On the left, a flowcell correctly positioned. On the right, the flowcell do not fit correctly between the fixations of the flowcell receptacle, creating a gradient blur-sharp.

19.2 The software is not working

Why this happens:

- The python code encountered a bug.

- There is a segmentation error because the number of objects is too important.
- The optical configuration tab does not work (black screen, impossible to change the WB, etc...).

Solutions:

- Try to restart the PlanktoScope
- Try to change values of the WB, check if you use commas or dots and restart the PlanktoScope
- Try to stand by a number of final objects around 2000~3000 per sample.
- Ask questions on the PlanktoScope Slack (see External links section)
- If you do not find any solution, flash the SD card of the PlanktoScope with the software by using BalenaEtcher

19.3 The pump is not working

If there is any problem with the pump, check that it is properly positioned. If it's not the problem, remove it and check that the pump tube is correctly positioned.

1. If you need to change the internal pump tube, you can take the pump off by turning it counter-clockwise.
2. Position the tube correctly.
3. To install it again, place it in the position shown in the image below until it clips, push it in and turn it clockwise until it snaps into the white socket.
4. Be careful to clean the grease afterward.

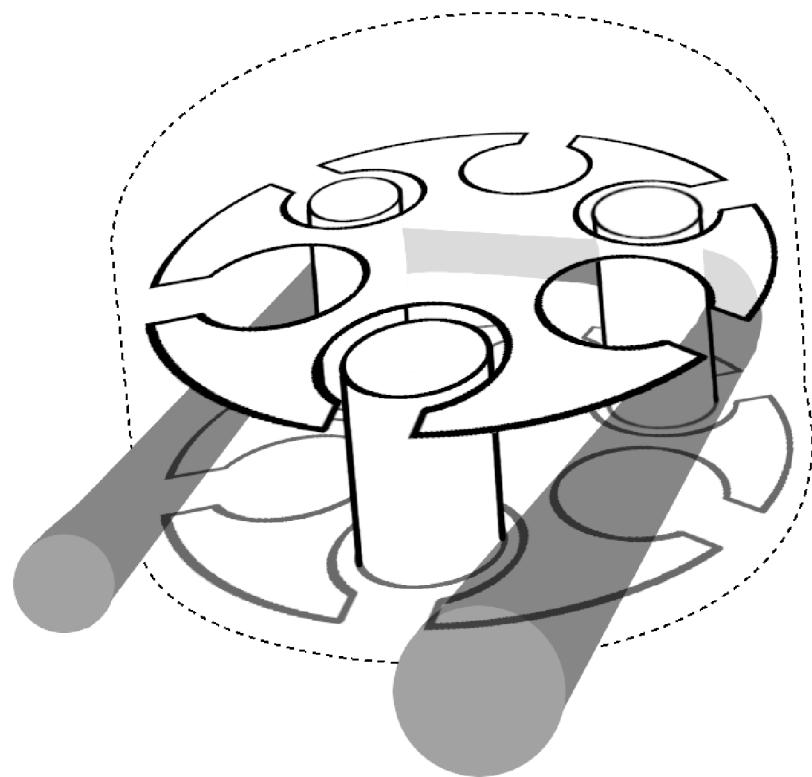


Figure 2: How the tube should be installed inside the pump.

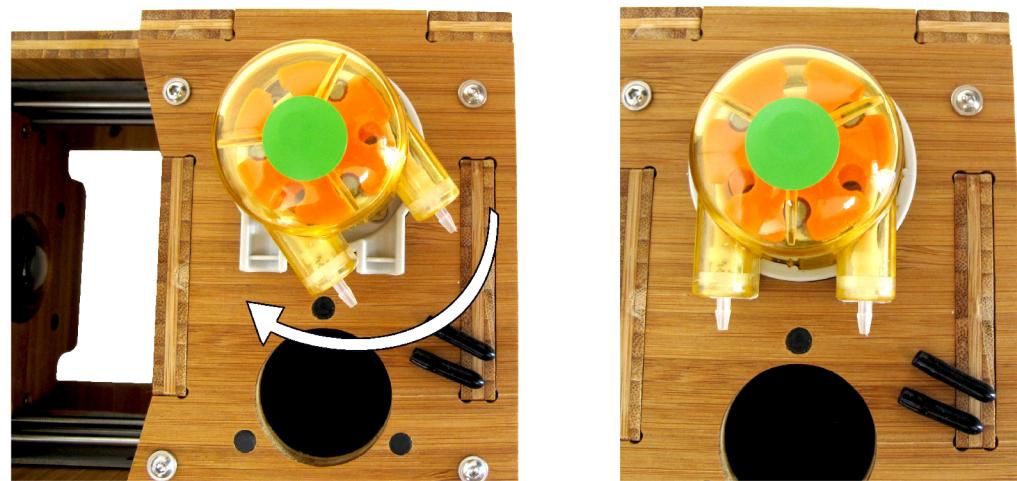


Figure 3: How to install the pump.

External links

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

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

Planktoscope complete assembly guide and complete documentations
<https://planktoscope.curious.bio/> (v2.5)

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

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

EcoTaxa tutorials:
<https://sites.google.com/view/piqv/piqv-manuals/ecotaxaecopart-manuals?authuser=0>
<https://www.youtube.com/watch?v=PS06ZS765tk>
<https://www.youtube.com/watch?v=RaWUqIoKk0E>