🌐 Planktoscope protocol for plankton imaging V.2

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LOVComplex

Ⓛ Lombard Fabien

DISCLAIMER

this protocols applies to the version 2.5 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/

IMAGE ATTRIBUTION

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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 it 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
200μm sieve
Squizing bottle
micrometer slide (or millimetric ruller)
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 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)

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

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

Created: Oct 06, 2022

Last Modified: Feb 14, 2023

PROTOCOL integer ID:
70911

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

Quick usage version

1   **Preparation**

1. Plug in the Planktoscope
2. Connect to Planktoscope's Wi-Fi ⇶↻ go to step #3.6
3. Type Planktoscope URL http://192.168.4.1:1880/ui/
4. Check WB (in OPTIC CONFIGURATION) ⇶↻ go to step #3.9
5. Put 20 mL sample, add the air pump ⇶↻ go to step #7.3
6. Check Focus (in OPTIC CONFIGURATION) ⇶↻ go to step #7.4

1.1   **Acquisition**

1. Fill sample details (in SAMPLE) ⇶↻ go to step #7.5
2. Fill acquisition parameters (in FLUIDIC ACQUISITION) ⇶↻ go to step #7.6
3. pump to drain sedimented organisms ⇶↻ go to step #7.7
4. START! ⇶↻ go to step #7.8

1.2   **Cleaning** ⇶↻ go to step #10

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

Plus, 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)

1.3   Analysis

1. Fill segmentation parameters (in SEGMENTATION) ⇌ go to step #8
2. Start segmentation
3. Back-up data ⇌ go to step #9
4. Import on ecotaxa ⇌ go to step #11

## 1.4   Shut down

1. Turn OFF (HOME)
2. Unplug the Planktoscope

Planktoscope, overview.

2   The Planktoscope is a frugal, microfluidic microscope designed with an open-hardware, open-software approach. It was conceived within the idea of equipping the thousands of 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.

Material:

The device and its different parts are shown in the following figure.
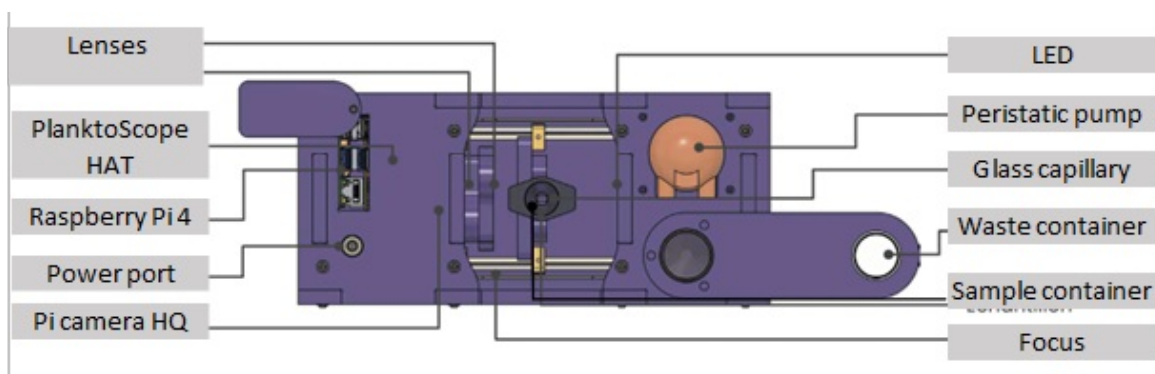
The PlanktoScope kit also includes a bubbler, power cable, waste container, falcon tube of tap water, syringe (containing the sample), sample holder, flowcell holder and flowcell.

The User Interface

There are several tabs on the UI 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
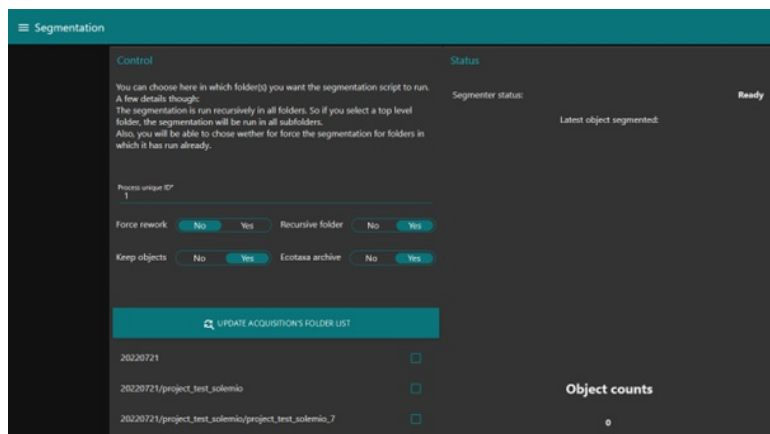
PlanktoScope.

We can also use the 'Hamburger Menu', situated in the top-left corner of the UI, to navigate between tabs.
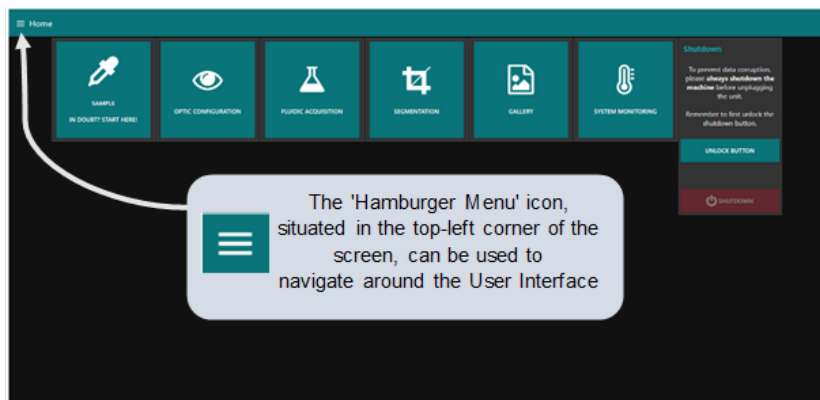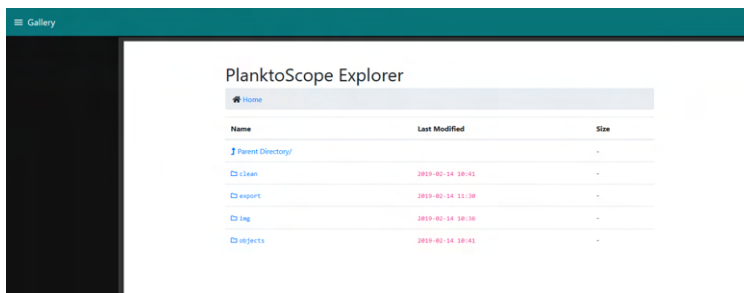

The 'Home' tab of PlanktoScope's User Interface
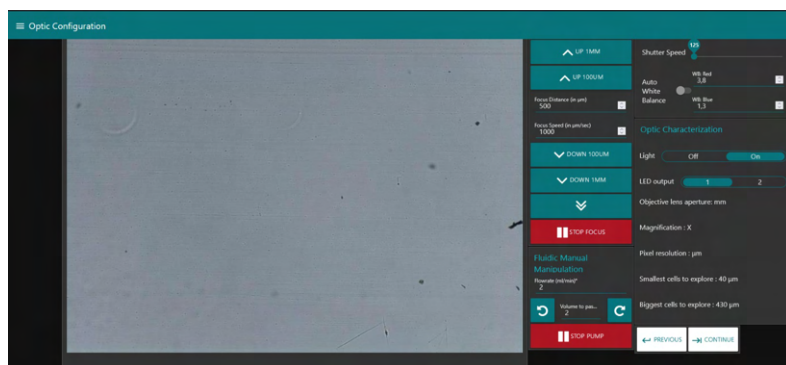
The "Optic configuration" page allows you to control the various features of PlanktoScope. You can focus, turn on the LED or start the pump.


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.

In the "Gallery" you can find the different files of the Planktoscope: the exports for EcoTaxa, the original images and the extracted thumbnails.
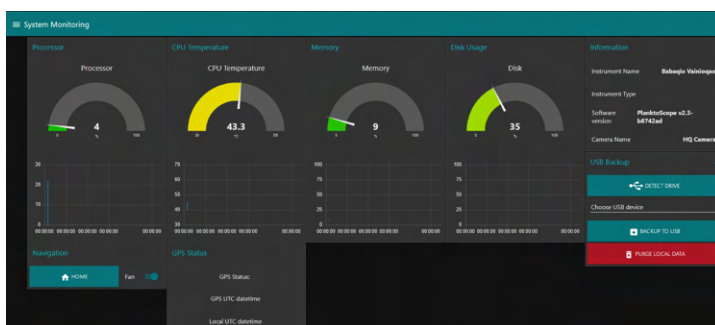
The "System monitoring" page allows you to check the correct operation of the device. You will not use this step in standard use.
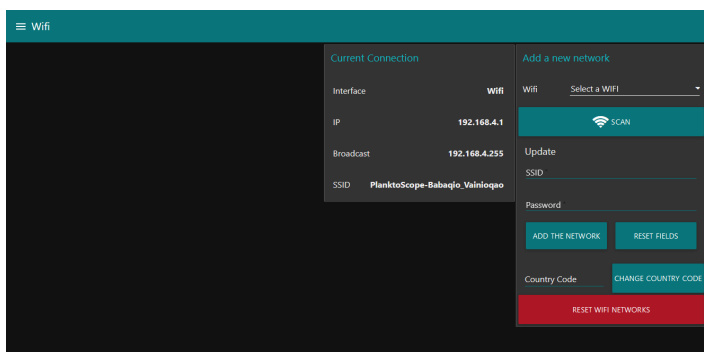
The "Wifi" page gives you access to the characteristics of the wifi generated by the PlanktoScope to which you will connect in order to control the device. You will not have to modify anything on this page. The procedure for connecting will be detailed later in this manual.
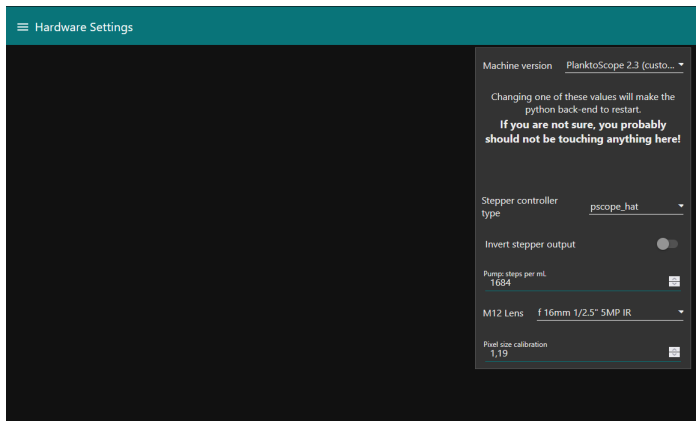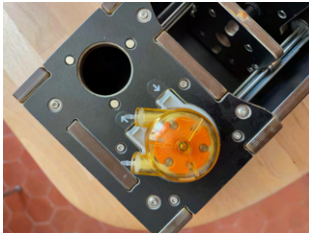
You will only need the "Hardware settings" page to replace the comma with a dot in the "pixel size calibration" box. Do not change anything else.

Initial connection and setup

3   Open the planktoscope box and check all the part first

3.1   if not installed, place the pump tube in place (the pump could be turned to be open). caution, clean the grease afterward
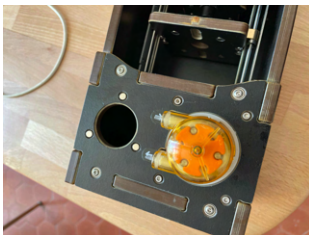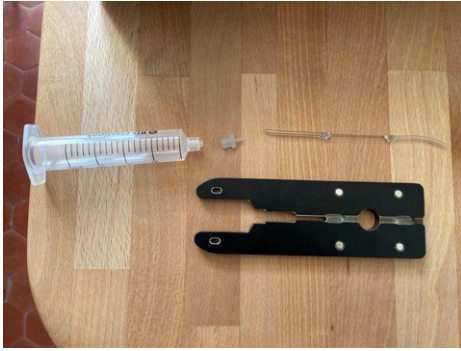

3.2   assemble the fluidic system

3.3   assemble the flow cell with care and step by step, note that a short length of the tube may need to be cut to get the right distance from the syringe to the flowcell
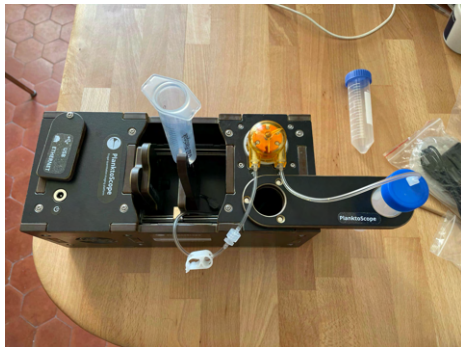
3.4

⚠️

to protect the flow cell during assemblage, first be gentle with it, and second place a spacer between magnets to avoid accidental breakage (here 3 layers of duck tape)

3.5 finally assemble all the system

3.6 Power your Planktoscope by connecting power cable to the power input and turning on the wall switch. Within 1 minute of turning on your PlanktoScope, you should see the LED flash once.

After a few minutes, you should see a new option for Wi-fi appearing on your computer. Connect to it using the password: "copepode".

For more information and alternative methods of connection, see the designer's Connectivity Tutorial here: 📎 PlanktoScope - Connectivity Tutorial (1).pdf

3.7 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):

http://192.168.4.1:1880/ui/

There are several tabs on the UI 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.


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

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

To test the PlanktoScope, navigate to the Optic Configuration tab:
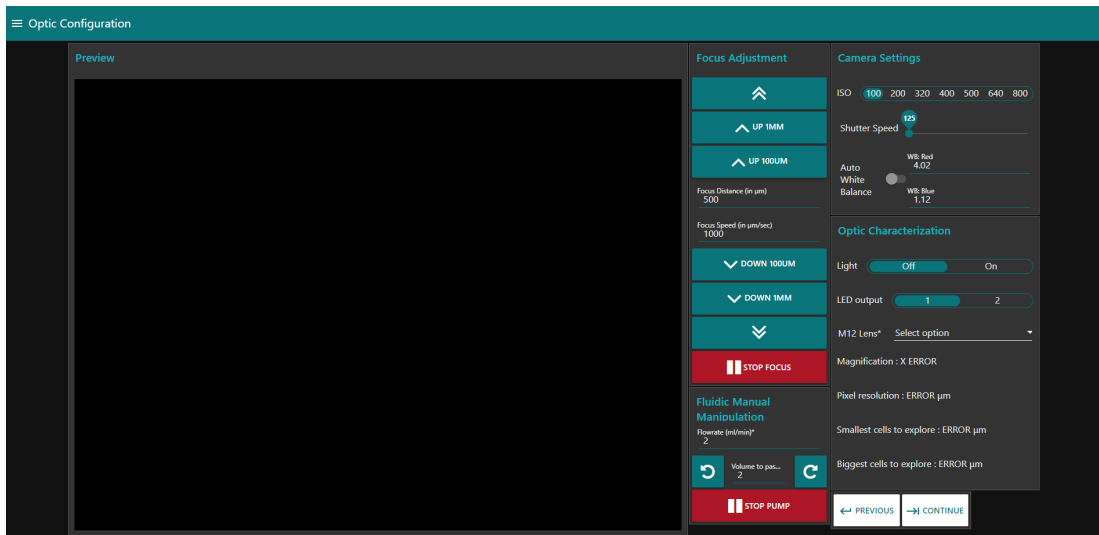
The Optic Configuration tab which can be used to adjust the camera settings. If only Preview is visible on your screen, the other options should be available below by scrolling down or by adjusting 'Zoom' on your browser (usually Ctrl + scroll UP or DOWN on Windows or command + scroll UP or DOWN on Mac).

a) Under Optic Characterisation, switch on the Light by clicking 'On'. You should see the Preview image turning from dark to light. The Preview image could be any colour so do not worry if yours does not show blue; it will be adjusted later.
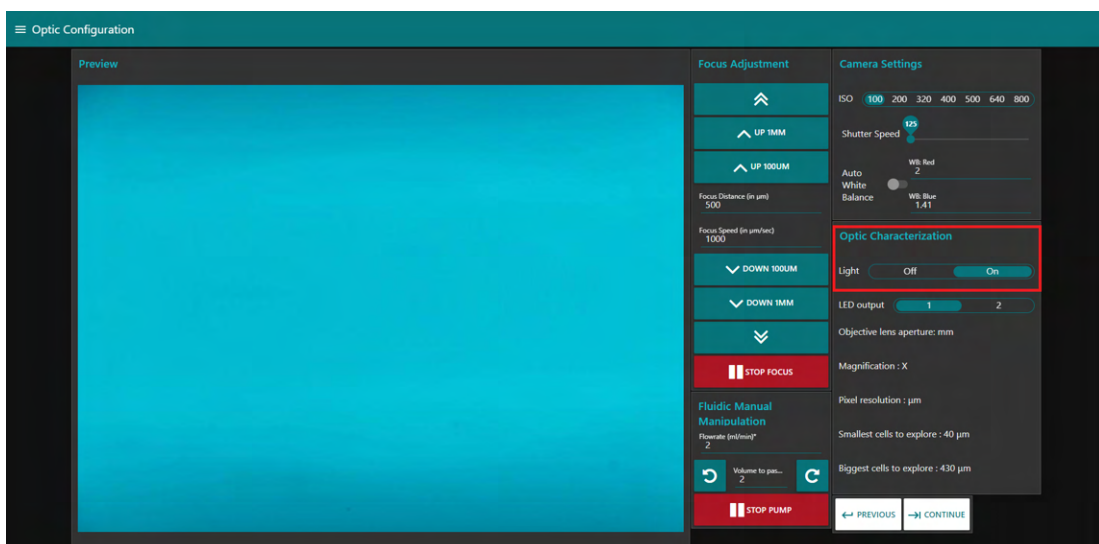

The red box highlights the location for turning on the LED. You will need to do this every time you use your PlanktoScope.

b) Under Focus Adjustment, click 'UP 1MM' and 'DOWN 1MM' to ensure focus buttons turn the focus motor. You should see the mount moving further from (UP) or closer to (DOWN) the camera.
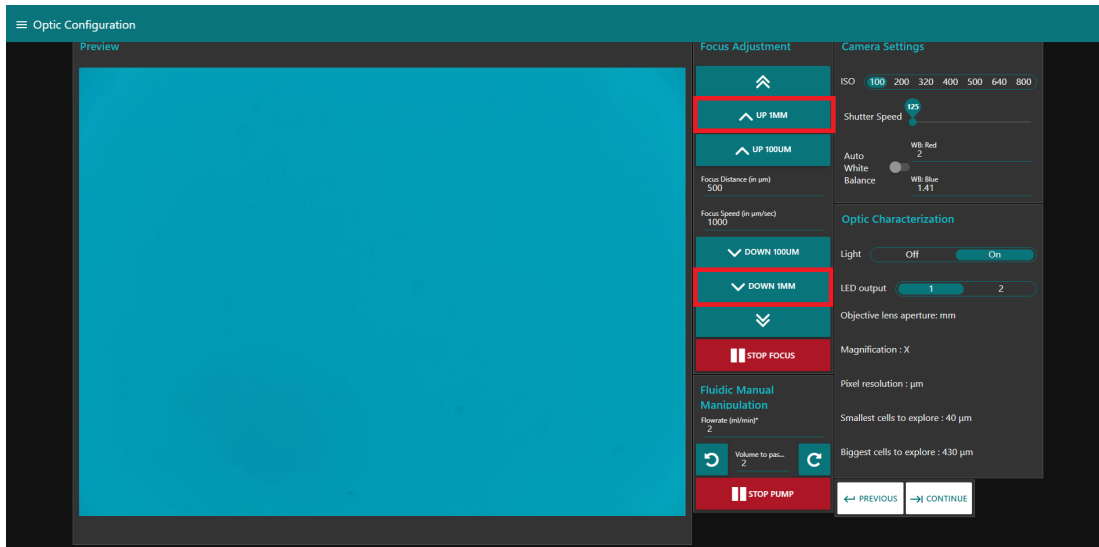

Red boxes highlight 'UP 1MM' and 'DOWN 1MM' that will move the Mount (pictured below).

c) Under Fluidic Manual Manipulation, click clockwise arrow to check that the Peristaltic Pump is working. You should see the pump rotating in an clockwise direction.
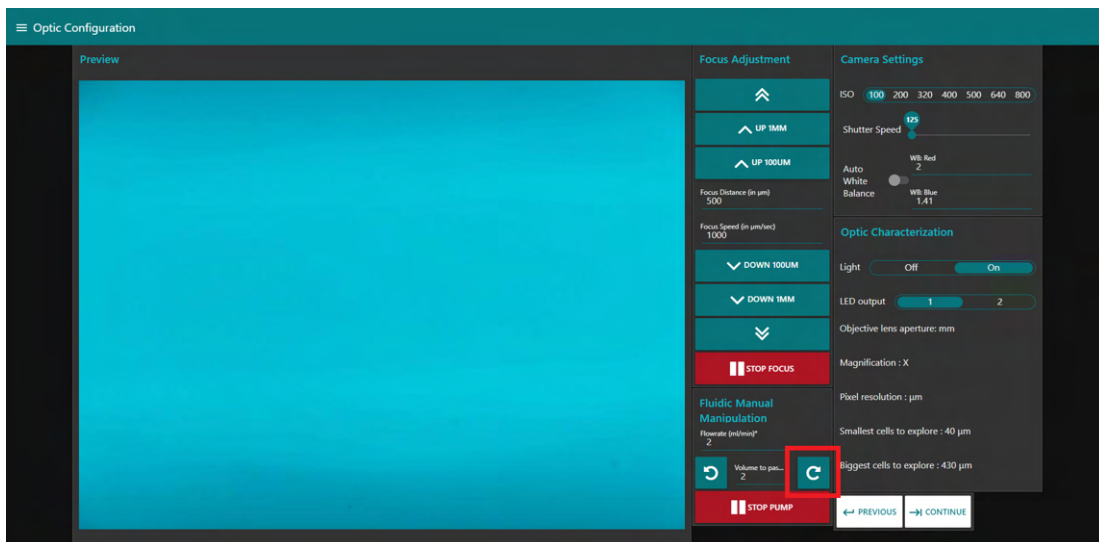
The red square highlights the location of the clockwise arrow that will rotate your Peristaltic Pump in the same direction.

d) Under Camera Settings, change the ISO value. You should see changes to the Preview image. After this test, Set ISO to 100.
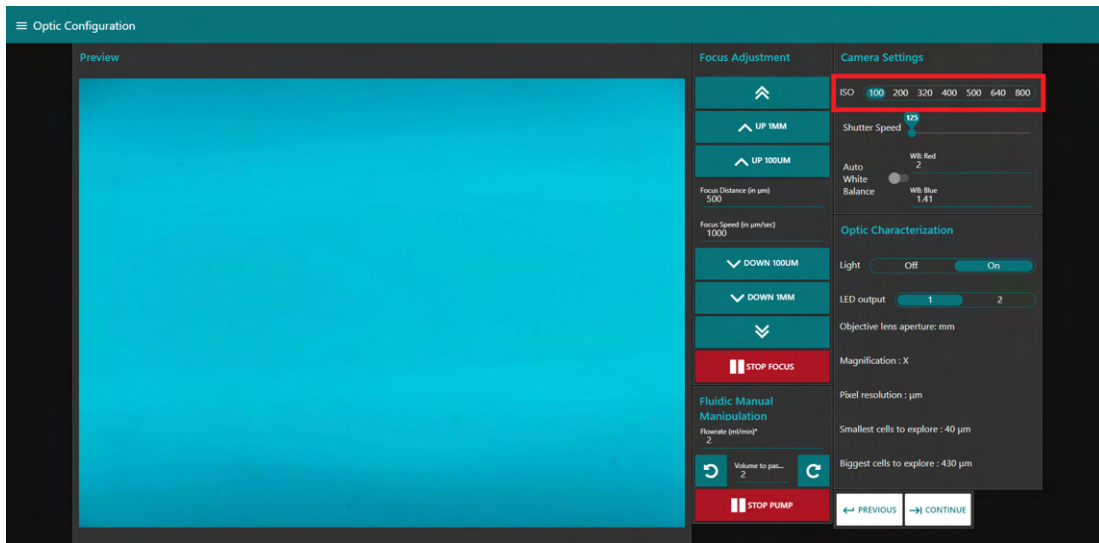
The red box highlights the location of the ISO setting. You should see your Preview image change colour when you adjust this setting.

Safety information

Make sure it is set to 100 once you have tested this.

3.9

Safety information

Mis-aligned lenses will create an inhomogeneous illumination and will create lots of artefacts if not corrected. We advise to check this every times you set back your planktoscope to use (closing the box may move the lenses)

Now we will align the lenses in your PlanktoScope. To do this:

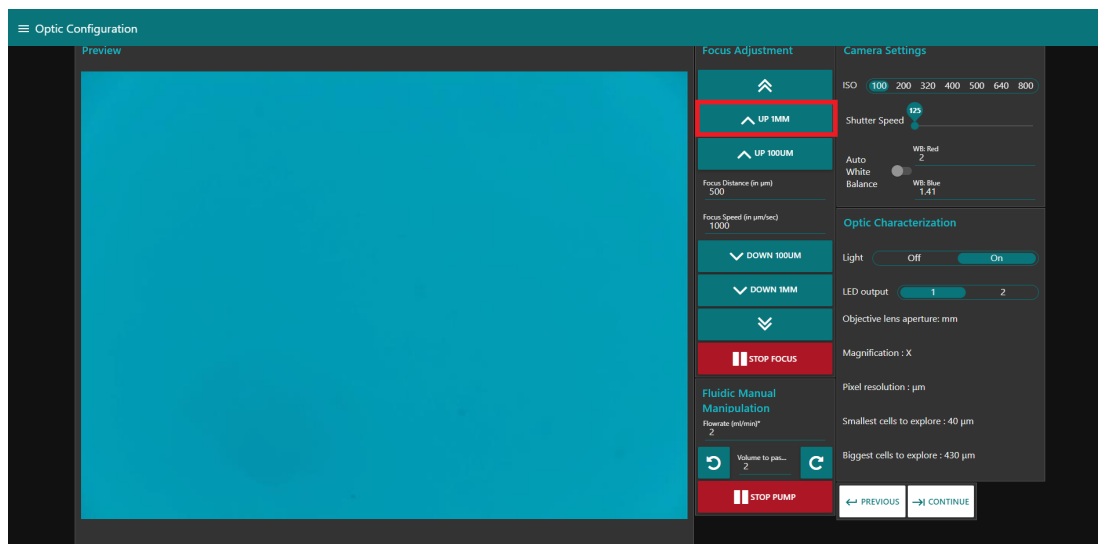a) Remove the Fluidic Path from the mount and gently lay to the side.
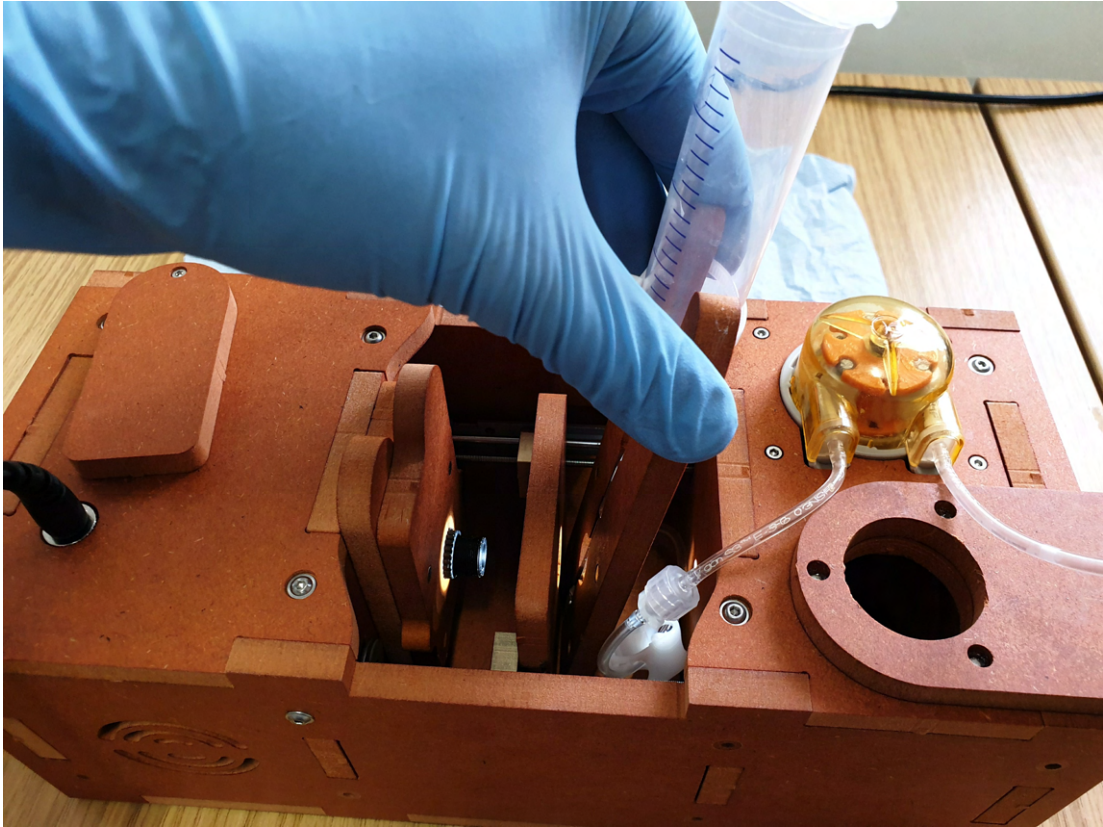
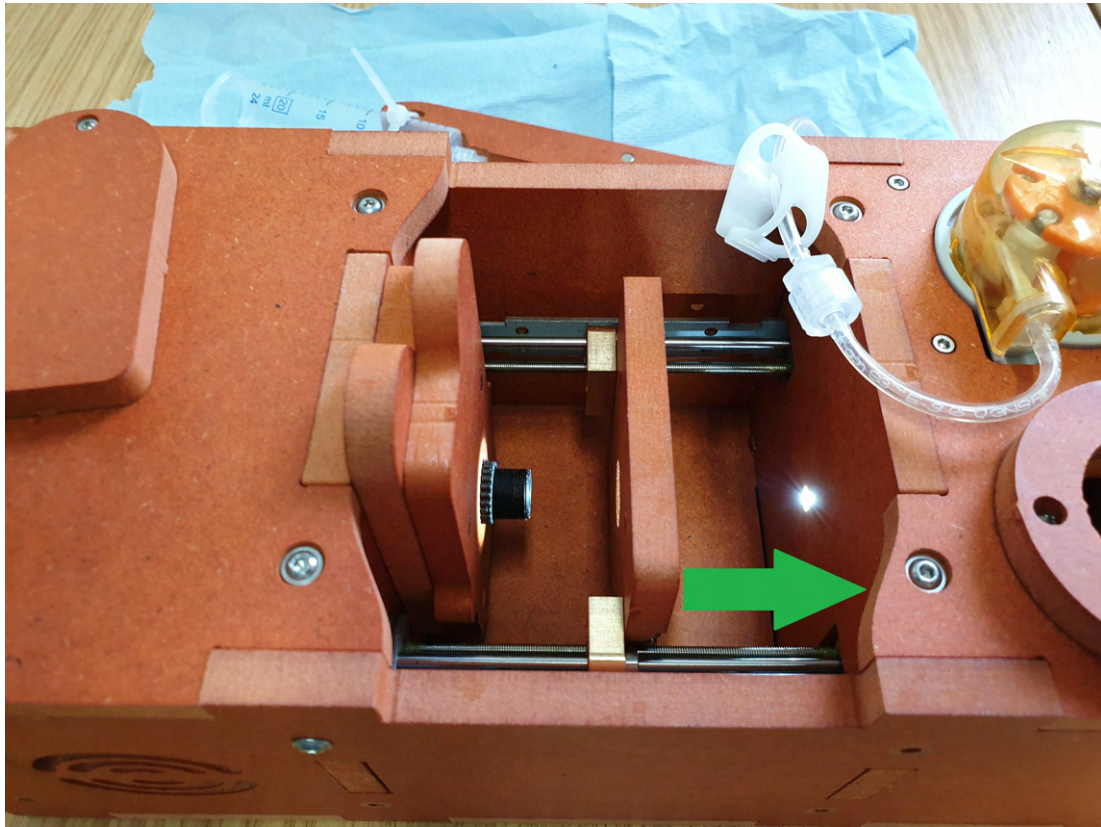Remove the Fluidic Path and lay to the side
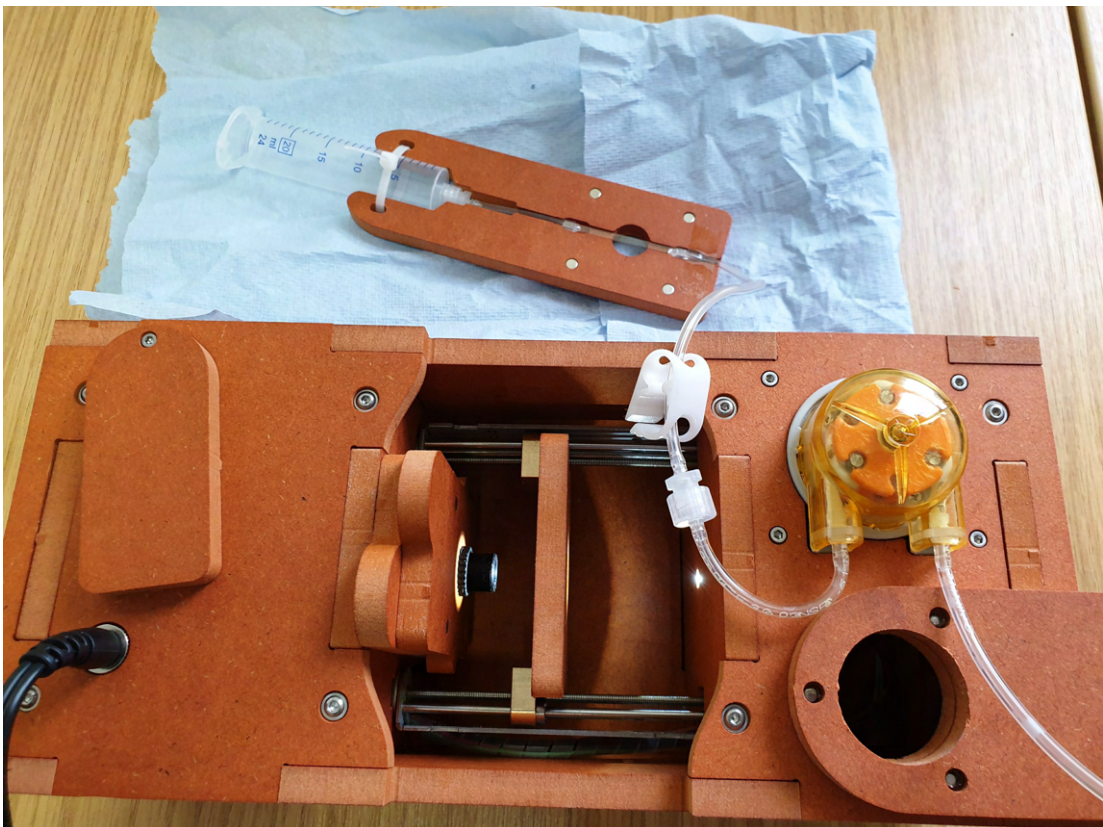
Remove the Fluidic Path and lay to the side

b) Make sure we have enough space to remove the lenses by clicking the 'UP 1MM' button under Focus Adjustment.
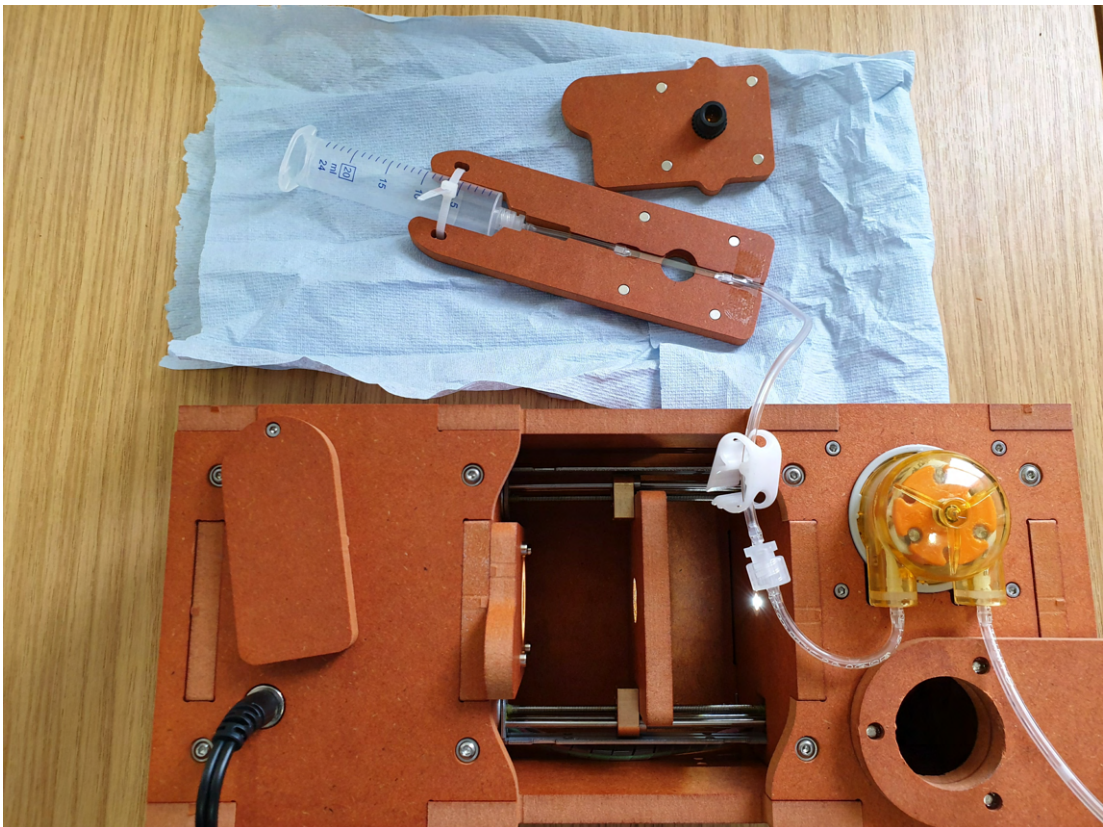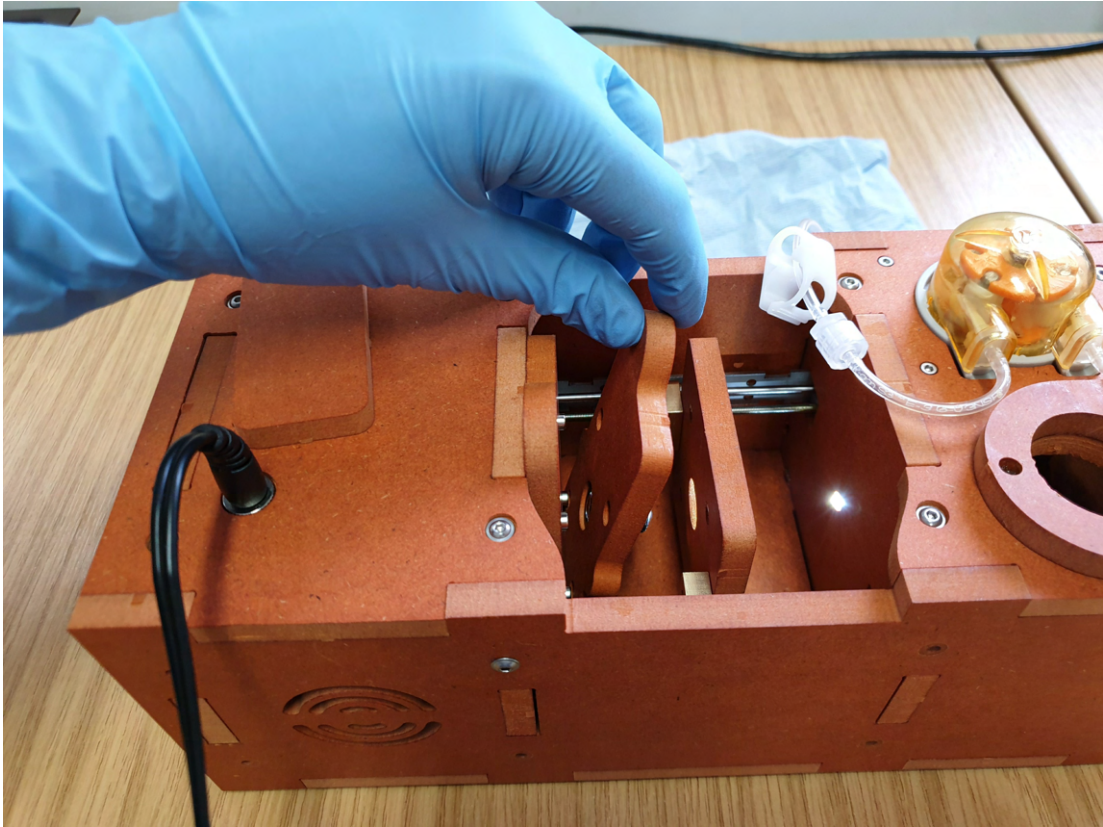

Red box highlights 'UP 1MM' button that will move the Mount away from the lenses.

Move the Mount away from the lenses using the 'UP 1MM' button to allow for outer lens removal.

c) Remove the outermost lens (16MM).

Remove the outermost (16MM) lens.

d) On the Preview image on the Optic Configuration tab, you should see a light spot surrounded by darkness. By moving the 25MM lens on your PlanktoScope, you can move the light spot on the Preview image. Try to get the light spot as close to the centre of the Preview image as possible.
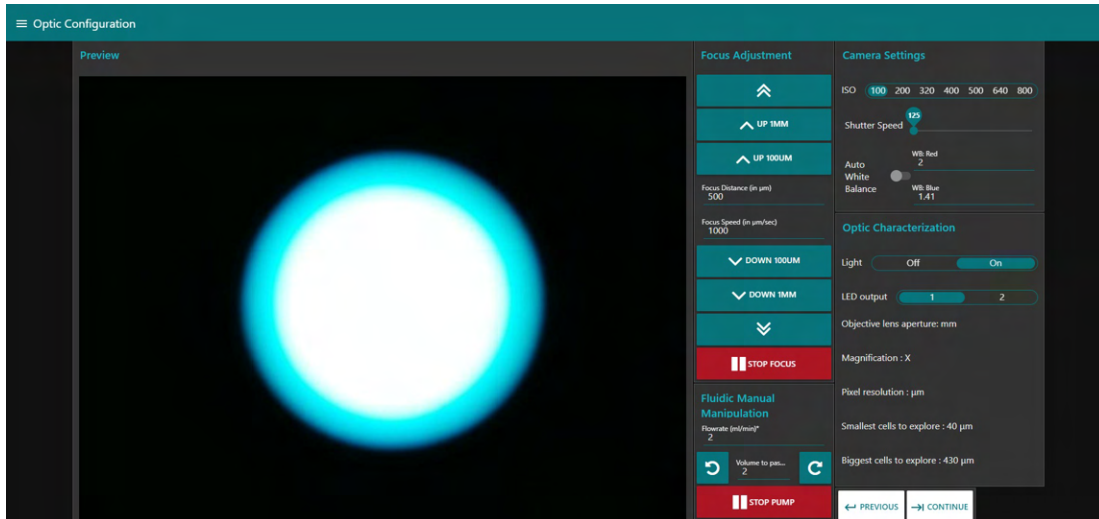
With the 16MM lens removed, your Preview image should resemble this picture. If the light spot is not centred, gently reposition the 25MM lens until it is as close to centre as you can get it.
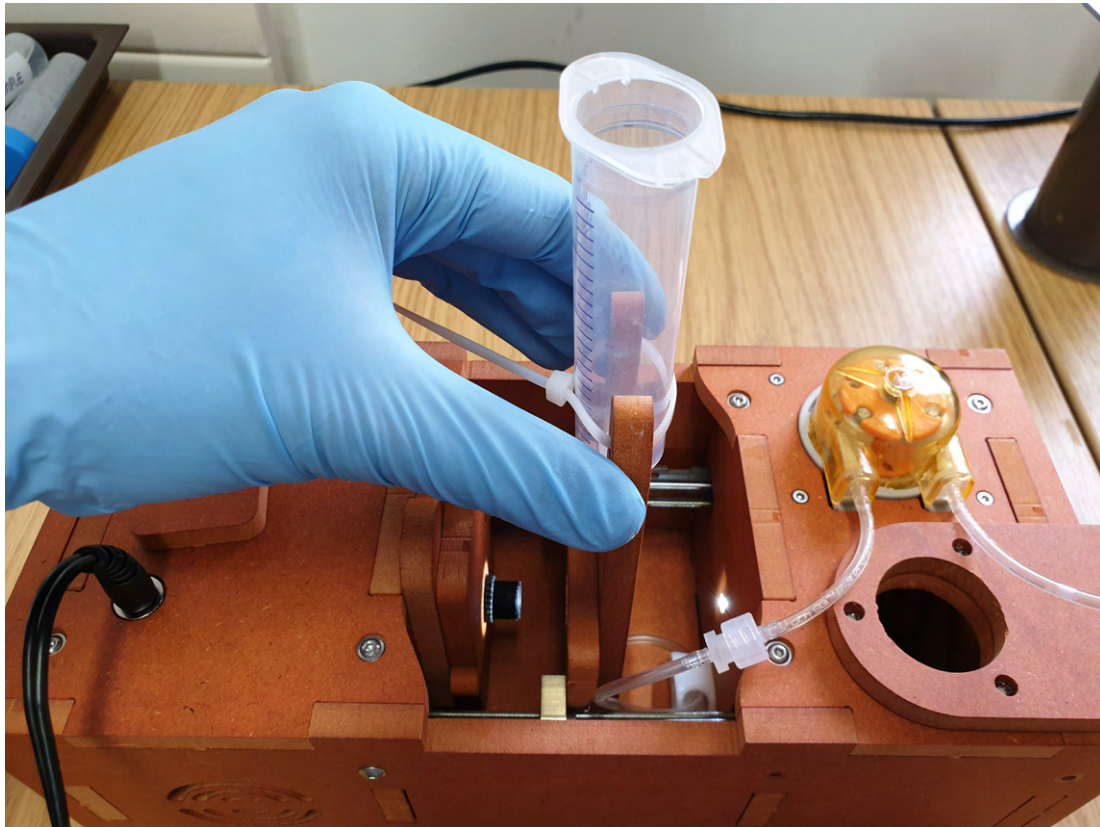
Gently reposition the inner (25MM) lens.

e) Once centred, place the outermost lens back to where you removed it from in step 3.

Place the outer (16MM) lens back into position.

f) On the Preview, you may see darker areas in the corners. To get rid of the darker areas in the corner, reposition the outermost lens (16MM) while holding the innermost lens (25MM) steady; the darker corners should disappear. Try to achieve homogenous light across the Preview image.

g) Place the Fluidic Path back into position.

3.10

Manually adjust the white balance of your PlanktoScope. 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 colour.

We need to achieve the Preview image colour that the Auto White Balance feature provides, without using the Auto White Balance. Not using Auto White Balance enhances the performance of the PlanktoScope over time (the camera will try to adjust it in between every images...)

To manually set to the White Balance, turn off the Auto White Balance and adjust WB: Red and WB: Blue until it looks white. Then switch AWB back on to see if it matches. Repeat this process until there is no colour change when clicking the AWB button.

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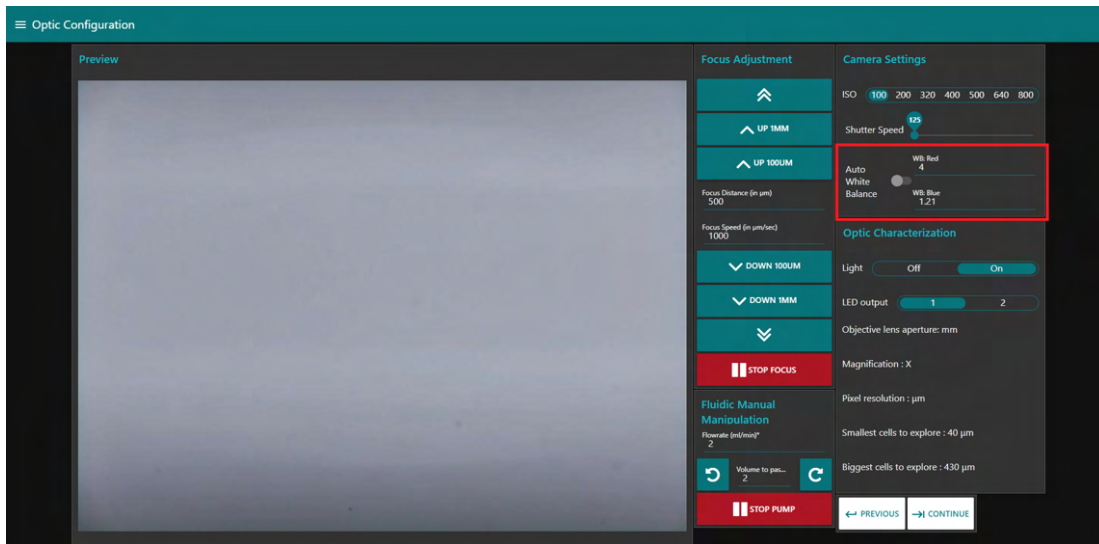
The red box highlights how to manually adjust the white balance of the Preview image. In this example, the correct setting was WB: Red = 4 and WB: Blue = 1.21. The AWB button should be set to 'off' once you have completed this step.

3.11

> **Safety information**
>
> Planktoscope image fluid at low speed.
> Not agitating your sample will let plankton to sediment and could even block the fluidic part. More importantly, the organisms concentration will be inhomogeneous, and because you will first get the sinking plankton, will lead your measurements to over-estimate true concentrations.
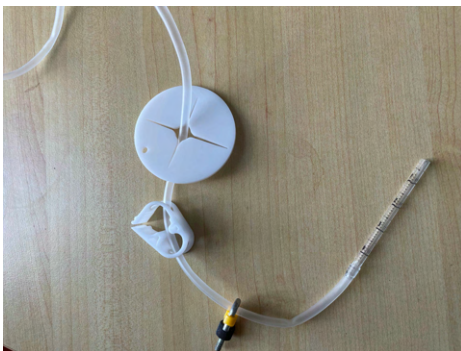
Setup the 'Bubbler': the flow of air needs to be adjusted to 1 bubble/sec approximately, bubbling the bottom of the syringe. We encourage adapting a rigid tube at the end to ensure the flexible part does not get aspired by the water flow
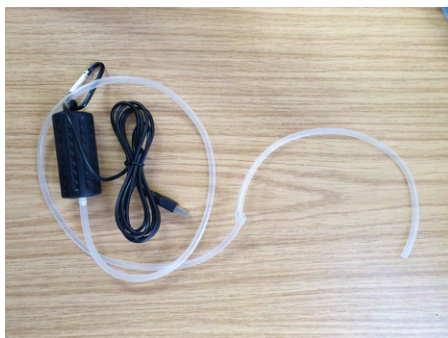
the bubbler

Tie knot to adjust air flow (basic way, not recommended)

examples of other possibilities to regulate air (choose preferred one) and adaptation at the end of tubing

Plug the Bubbler into one of the USB ports on the PlanktoScope. Place the tubing into the Syringe so that it reaches the bottom. Affix the tubing to the Syringe using an elastic band, string or similar.
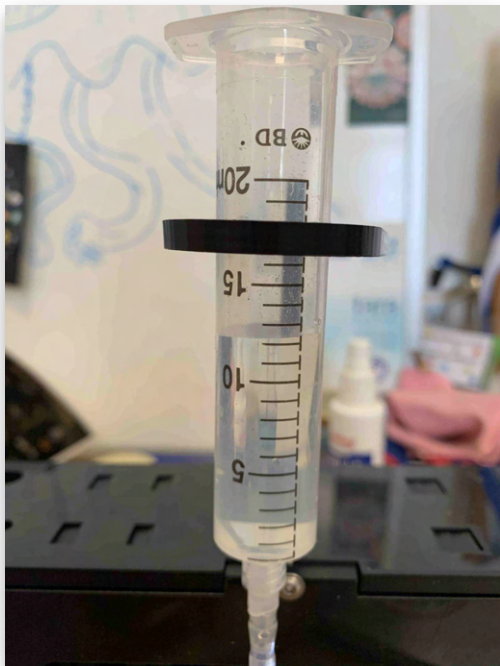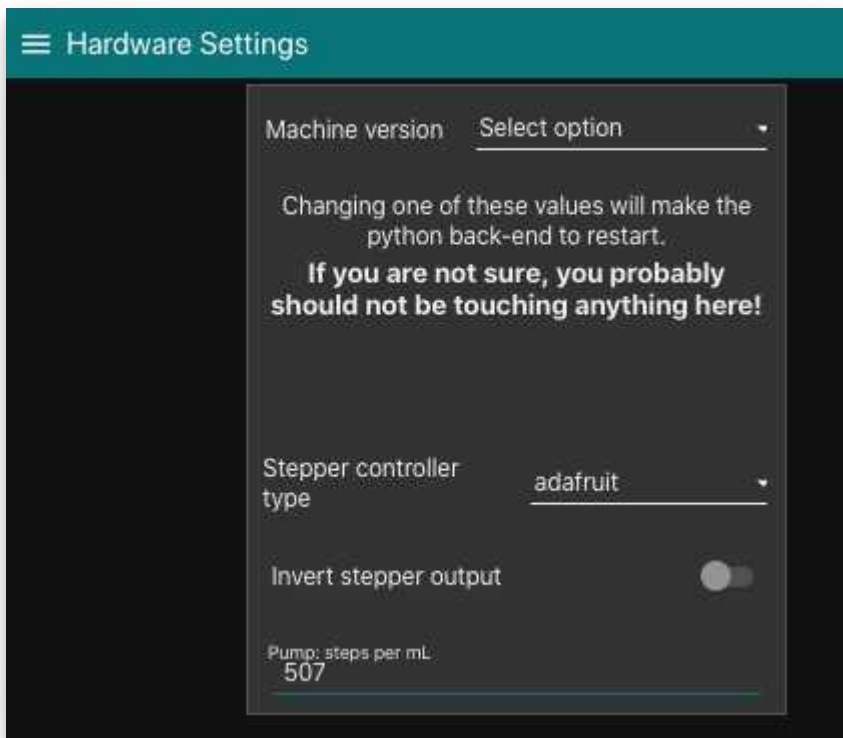
Calibration

4   **Pump calibration:**

4.1 -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)

4.2 -then on the hardware setting, note the "pump step per ml" parameter (old step)
-calculate the "calibrated" pump step per ml such as = 10*old step/ X
-replace the "pump step per ml" parameter with this value

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

5   Size calibration:

5.1   - tilt the planktoscope on the side (camera on the bottom)
- 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
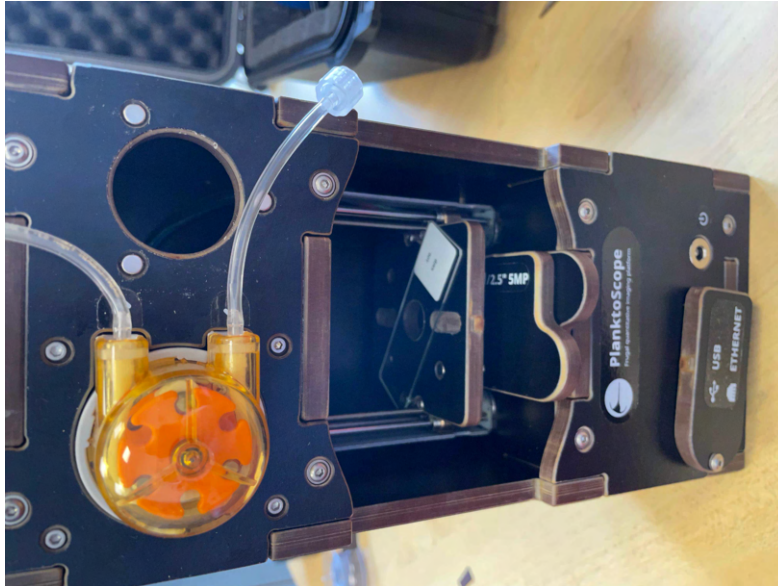
tilted position for calibration
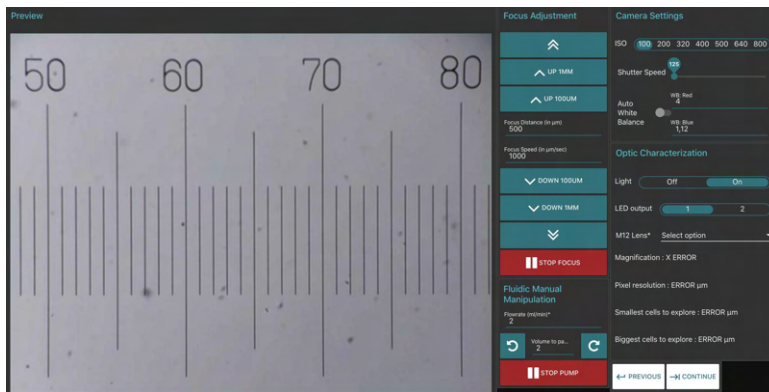
focusing on the scale

5.2 -take few images (select the test or culture mode in sample tab), goes on acquisition and put 1 or 2 images

example of metadata entered in "sample" page

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

5.3 -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) (s**ee section 7 for communicating with your planktoscope and downloading the raw images**)
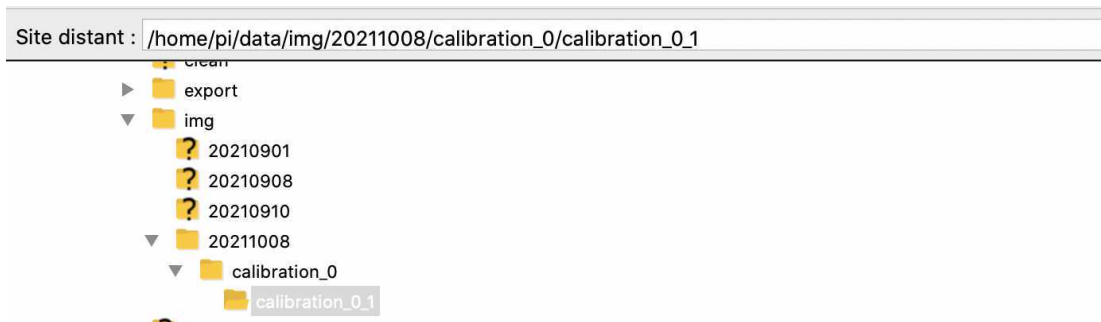
downloading resulting images (using Filezilla)
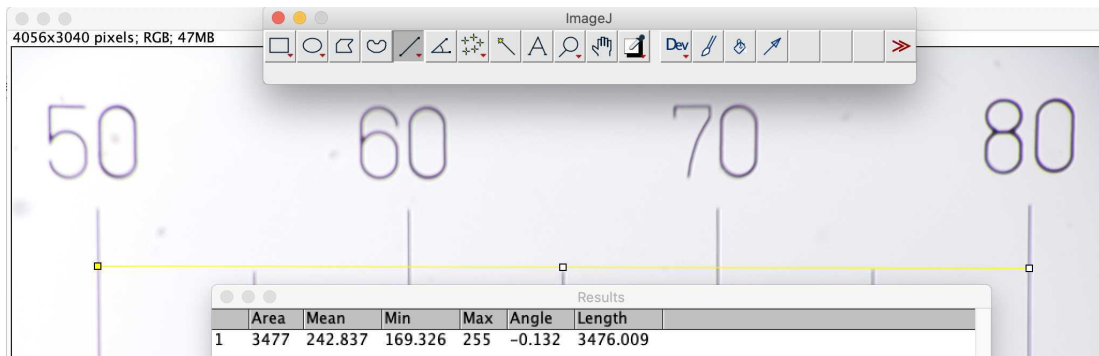
using imageJ open your image (File/open), draw a line as long as possible (here 3mm on the scale) and measure it (analyse/measure). The line is 3476 pixel length (i.e. one pixel is 0.86 with this example)

5.4 -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)

5.5 Enter the calibrated pixel size value in the hardware setting
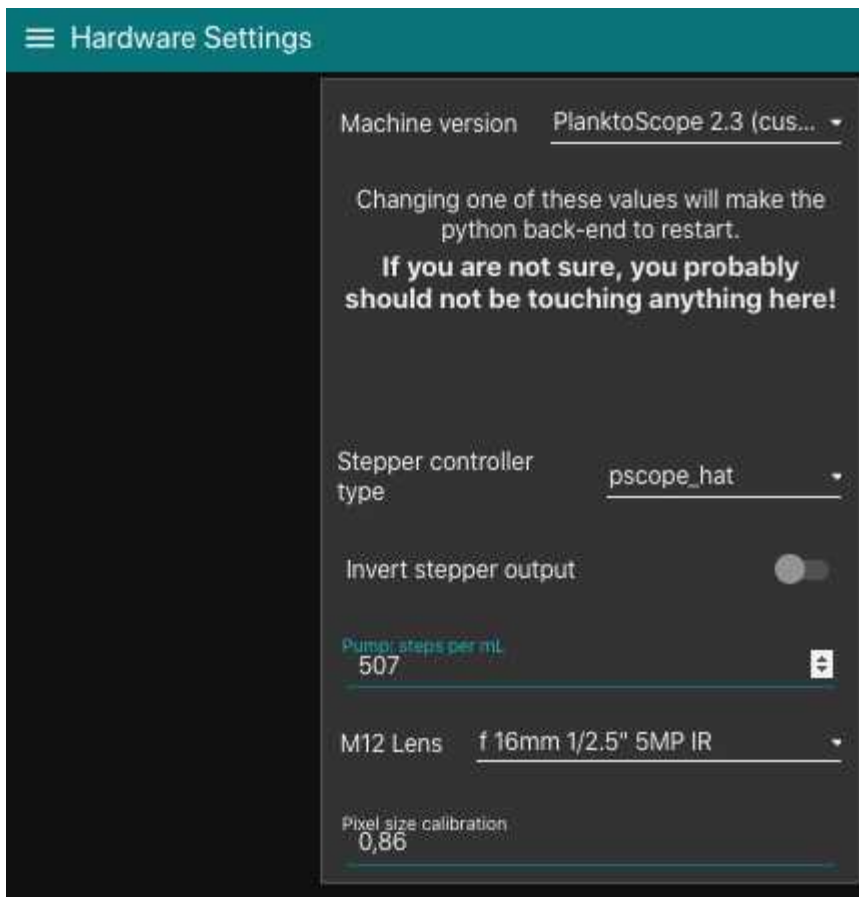
Known bug: **note here that the comma should be replaced by point at every restart of the system**

Get your sample

6 Use a net to collect plankton

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 informations to get to quantitative sampling (see step 5.4)

6.1   Get the content of the collector.
Pass the volume through a 200µm sieve

larger organisms may clog the flowcell

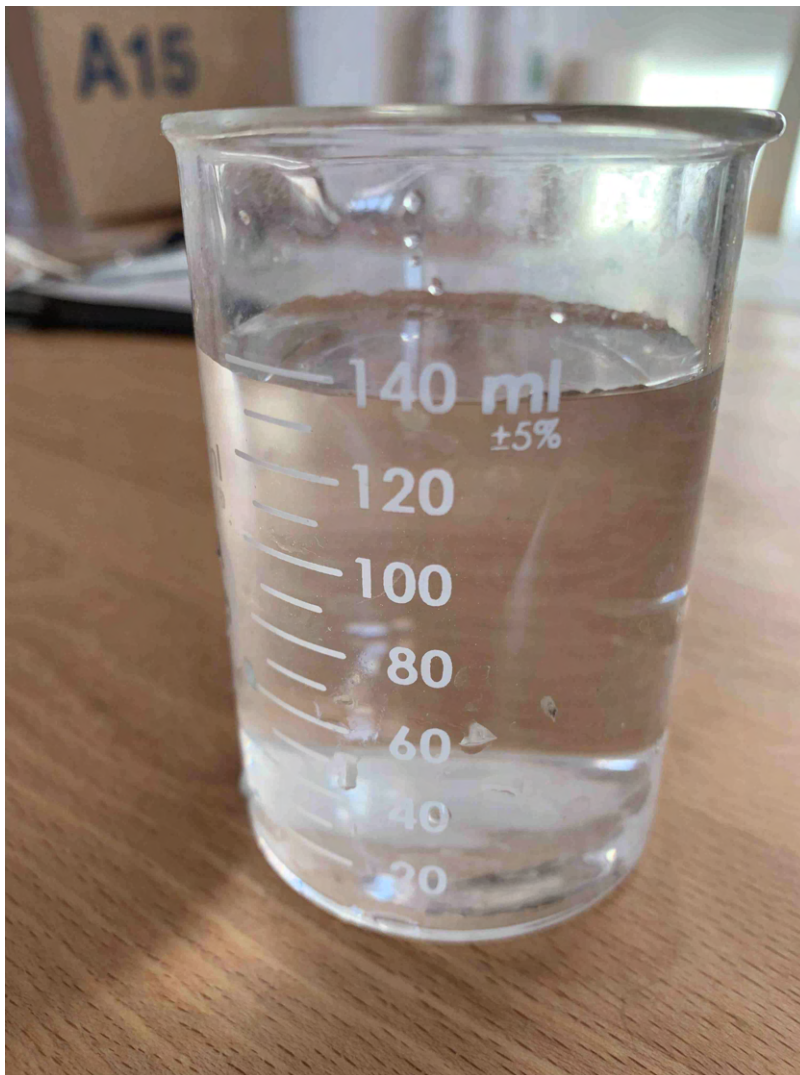filtration through a 200µm mesh

detail of the (home made) filter

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

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

<div>

Safety information

Those are critical informations to get to quantitative sampling

</div>

concentrated final volume of plankton
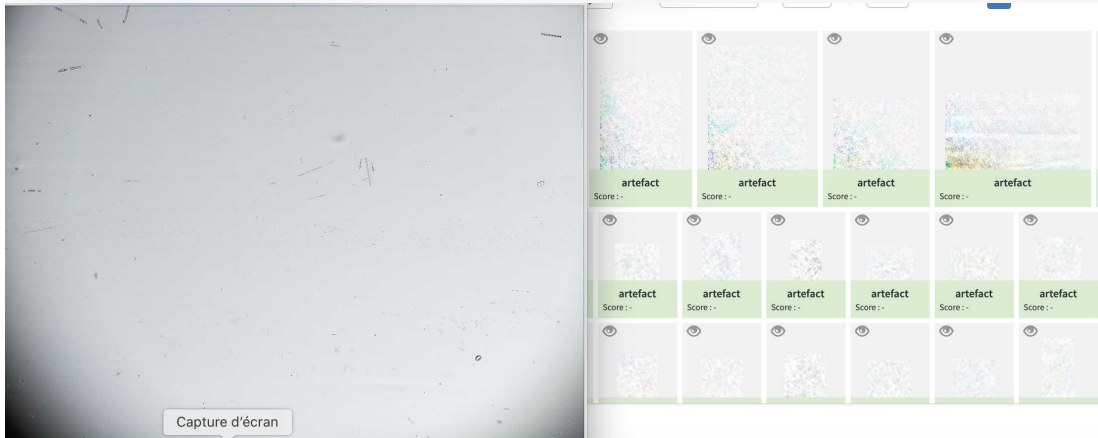
Pass the sample on planktoscope

7   assemble and start the planktoscope (see ⮌ go to step #3)

7.1   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)

unaligned tube lens could create strong inhomogeneous background in final images, leading to creating lots or 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

7.2 Fill the sample on the sample holder. 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).
Replace the full sample holder and reconnect it to the pump, open the stopper, place the bubbler and adjusts it flow.

7.3 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#1**: 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; **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)

> **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

7.4 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 in 7.5**

> **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

7.5 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 #6.1** ; **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 **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

- 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 of sample recovered after all the steps of concentration or dilution.

- If dilution have been done, note the "dilution factor" (if not, write "1").

Dilution factor if a dilution has been done in **⇌ go to step #7.4**

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

7.6 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)

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)
Getting lower numbers

-**Volume to pump** is the volume pumped in between two images: it should be large enough to : avoid taking two time the same object in photo; avoid large sedimentation in the fluidic system; avoid objects to stick on the flowcell. We recommand to test 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 object moving while imaged.

The Planktoscope is operation 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 use 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 bellow)
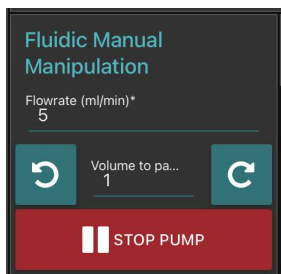
diatom imaged when moving

copepode nauplius moving while imaged

7.7 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)

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

Segment the acquisition

8

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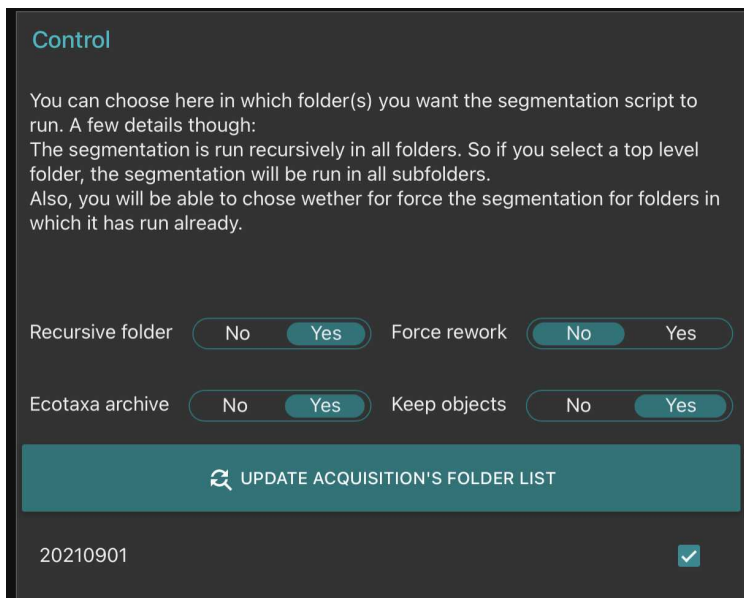
- scroll down and clic on start segmentation
- Wait for the segmenter status to turn to "done"

Download the results

9   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://192.168.4.1 (note images were taken with a previous version, the adress does not correspond to images)
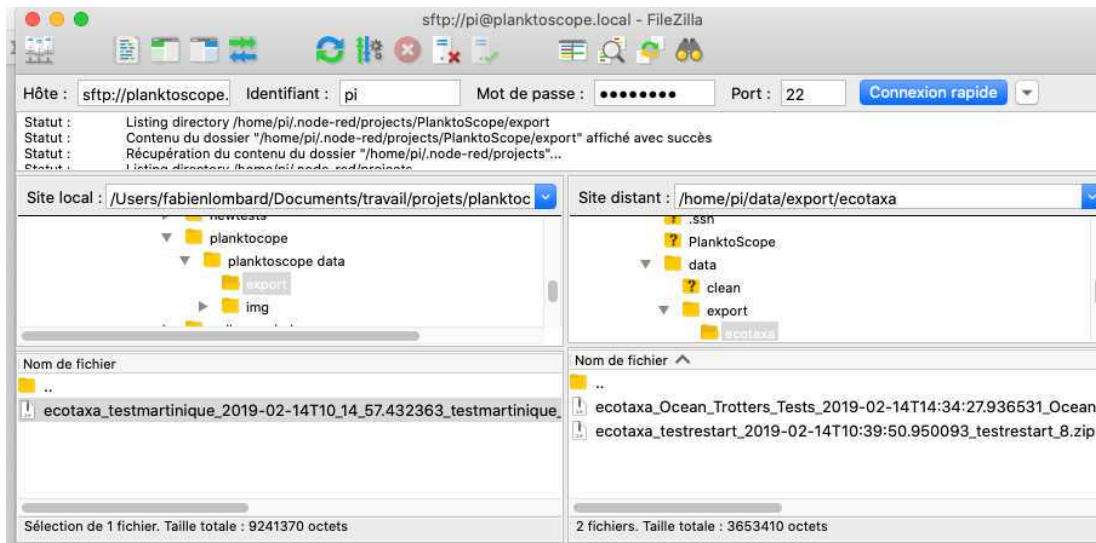Username: pi
Password: copepode
Port: 22

- 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 substraction, masks of the different objects etc) are in /home/pi/data/clean

Final vignettes are in /home/pi/data/objects

Clean the planktoscope

10
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 #7.8)
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

Upload your images on EcoTaxa

11 **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 *

| John | | Doe |

Email address *

| john.doe@foo.edu |

Password *   Password confirmation *

| Type your password | | Retype your password |

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 *

| Select from the list or type a new name | ▼ |

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   Cancel

11.1   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

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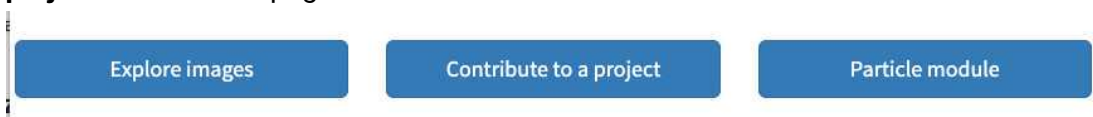
11.2   **Needs to be done only once:** basic rights don't include the "create project". As a protection against bots, To create a new project and upload images in it, please contact the user manager(s):

- PIQv (piqv@imev-mer.fr)

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

without the right to create projects

with the right to create projects

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

**11.4** upload the ecotaxa archives (see step 6-7) on the EcoTaxa ftp

using Filezilla (see) 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)

11.5 In your project/ on your project options button, select import images and metadata
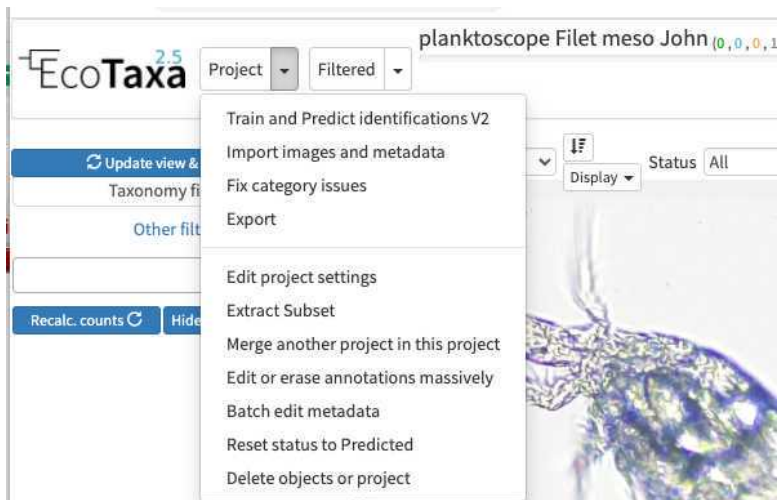
11.6 locate your file on the ecotaxa ftp folders and import it (only works for one zip file at a time for now)

How to use efficiently ecotaxa

12 **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

- Define a person of contact (mandatory)
- Define what pre-trained Deep Learning features to use on your project (we recommend to use «Planktoscope_2022-09 » unless you see a more recently trained model on planktoscope image)

- 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 #11.5), or just view the project (both validated and non validated)

12.1 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

taxonomic filters

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)
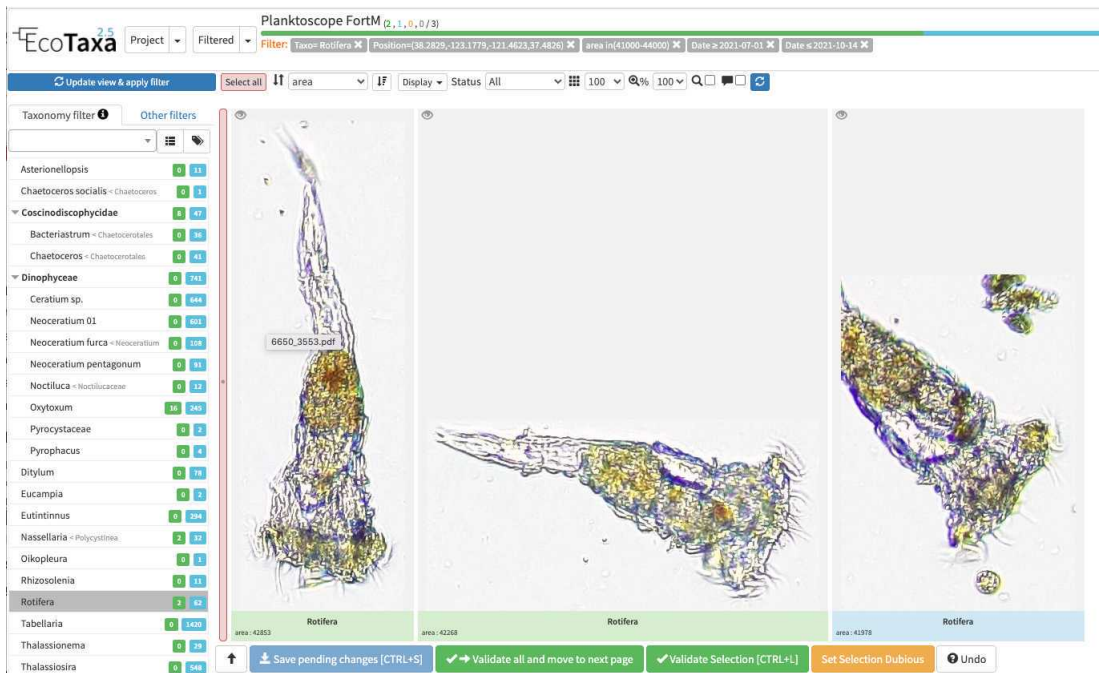
quickfilters are pretty useful since you can sort objects by specific values (eg. mean saturation below) to quickly observe objects that have here lots of chlorophyll (ps. you can revert the sorting order of those filters by ascending or descending order)
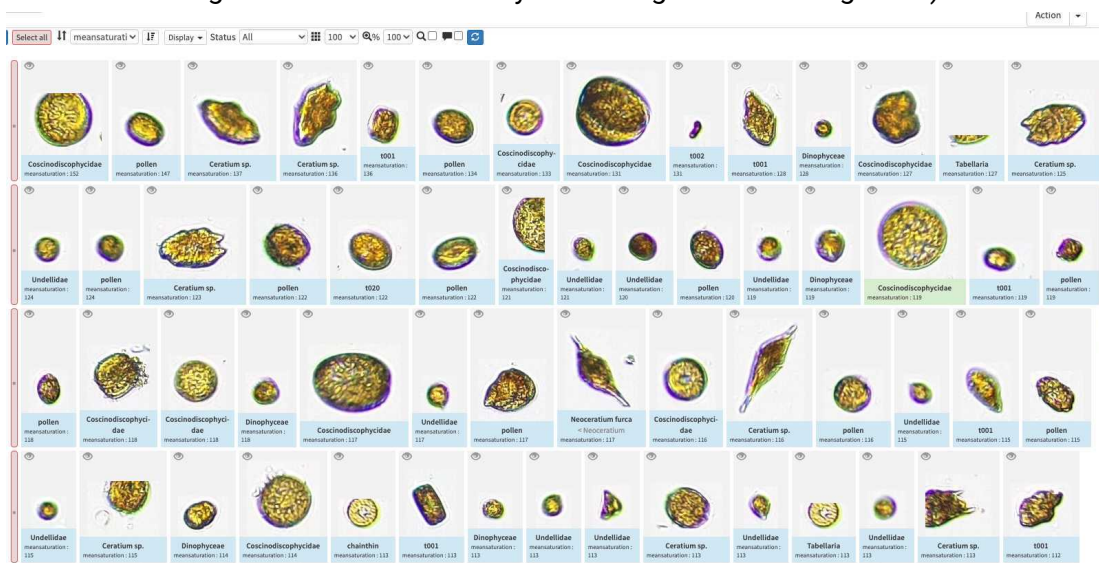
12.2   **The different validation "states" in ecotaxa** and how to validate
image arrives in EcoTaxa with the status "unclassified" (grey surrounding of the image)
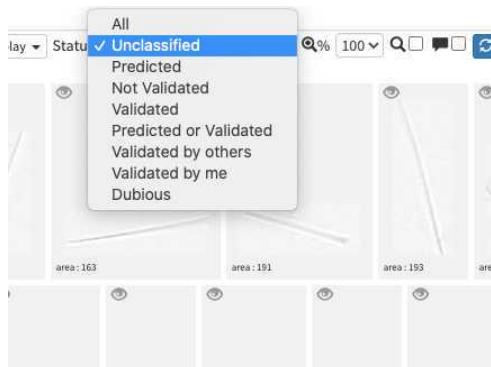
However they could be also set as "predicted" (blue surrounding; classified automatically by taking as example one pre-existing project), "validated" (green surrounding; checked and annotated by a human), or dubious (orange surrounding; checked and annotated as dubious by a human)

-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
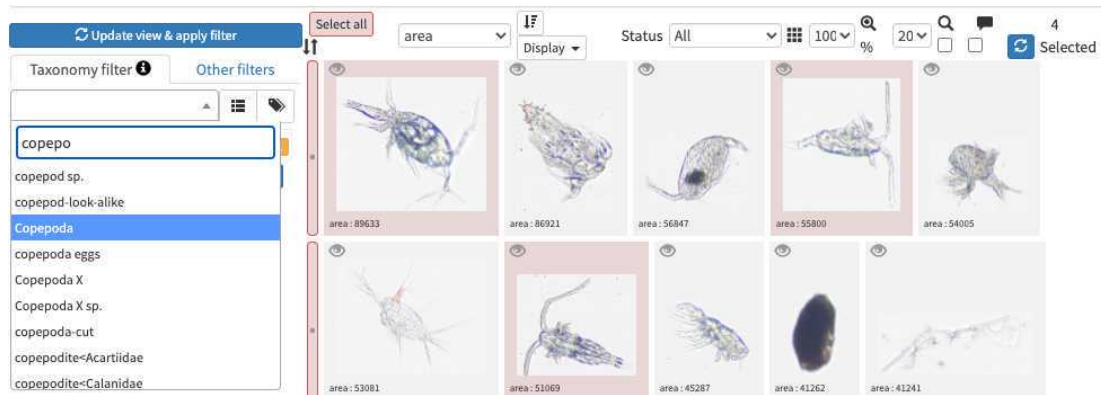
typing "copepo" brings several results
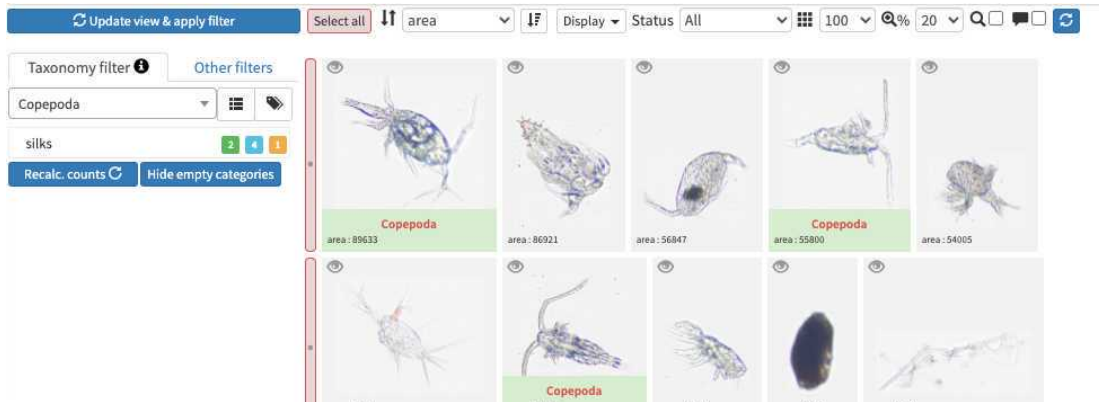
once validated the name appears in red below the images
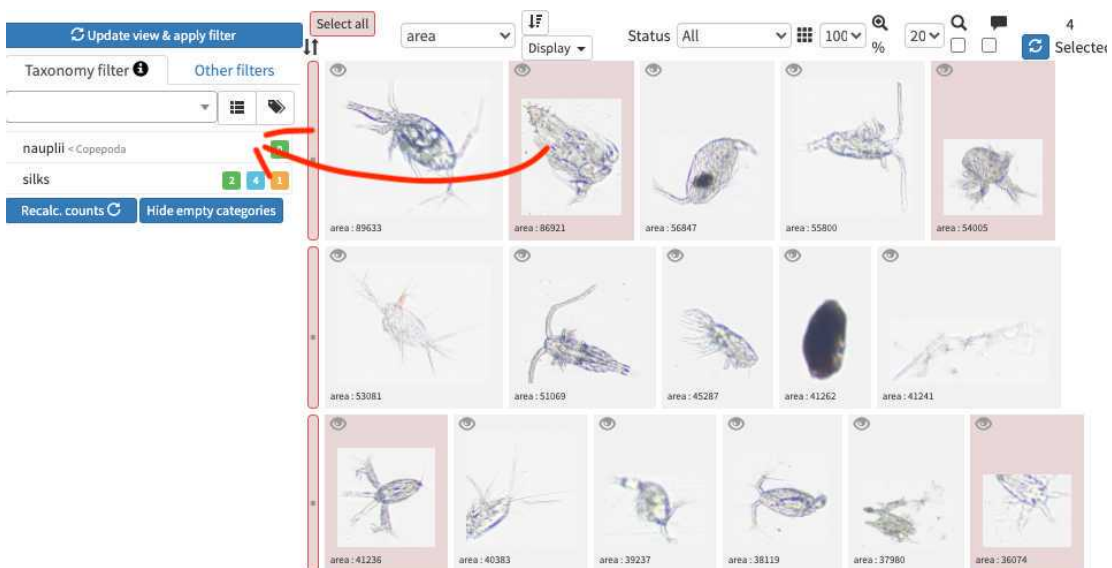
sliding into existing categories also works

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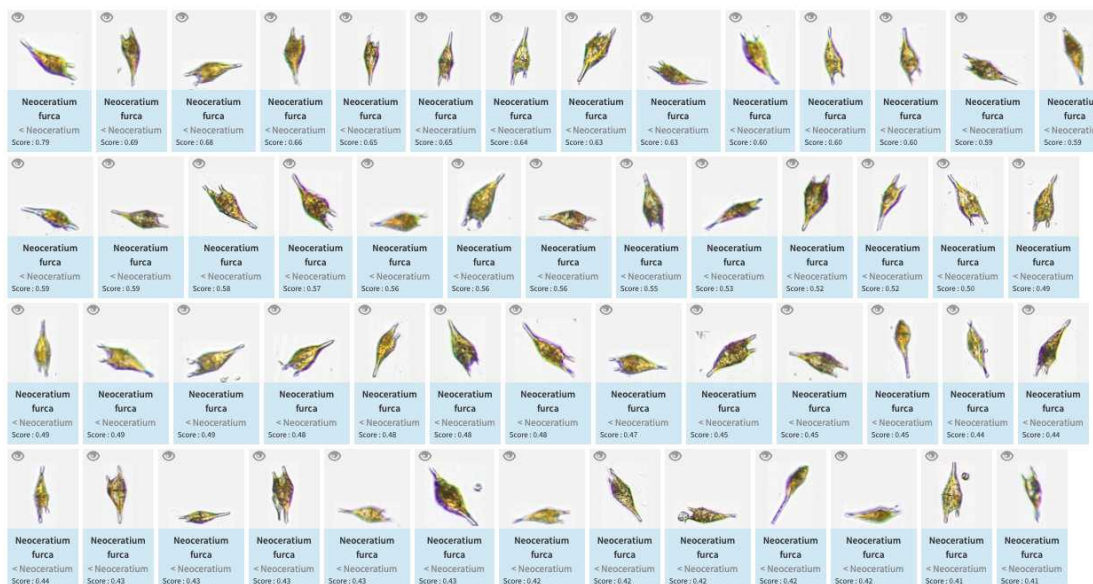
example of (well) predicted objects which would be easily validated

12.3 **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"
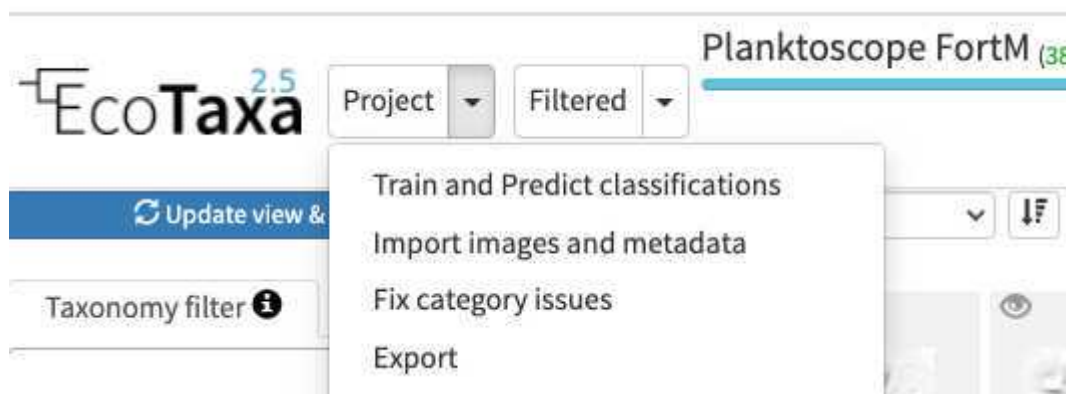
- You can select any of the pre-existing project (including your own project) as a template for image recognition. By experience, using another project is only a first-aid, but won't replace prediction on images that you acquired with the same instrument/ same location / same plankton communities
- Note 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 :
- #6818 - MOOSE-GE-2022_tests_ID_vignettes (Med sea; Processed with current segmenter; Fully validated)
- #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*)
- #6765 - _Planktoscope Reference (Processed with other segmenter, *works only with adding Deep Learning features into play*); Fully validated)

PREDICTION: Choice of Learning Set data source

Next: Choose objects in selected projects

A Learning Set AKA Training Data Set is built using validated data from one or several projects.
Inside these projects, you will be able, in next pages, to specify:
  - Which categories you're interested in predicting.
  - How many objects you want to use as reference for the prediction.
  - For these objects, which features are relevant to the prediction.
This Learning set will then be used for running the prediction task. ❓

Filter on title : [ ] # Matching features ≥ 10 Instrument : [ ] 🔍 Filter

Project deep features model: flowcam.
Model is usable ❓

# - 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

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.

Make categories appear like in:
[Project search ▼]

(id)	⊟	Source (validated) category	# source	% source	# learning set	Appear as category
(85008)	☐	artefact	13036	79.4 %		[▼]
(92010)	☑	silks	998	6.1 %	500	[▼]
(85079)	☑	multiple < other	335	2.0 %	335	[▼]
(85352)	☑	chainthin	198	1.2 %	198	[▼]
(92767)	☑	nauplii < 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 ☑	angle ☑	area ☑	area_exc ☑	bounding_box_area ☑	bx ☐	by ☐
circ. ☑	circex ☑	convex_area ☑	depth_max ☐	depth_min ☐	eccentricity ☑	elongation ☑
equivalent_diameter ☑	euler_number ☑	extent ☑	height ☑	label ☐	local_centroid_col ☐	local_centroid_row ☐
major ☑	meanhue ☑	meansaturation ☑	meanvalue ☑	minor ☑	perim. ☑	perimareaexc ☑
perimmajor ☑	solidity ☑	stdhue ☑	stdsaturation ☑	stdvalue ☑	width ☑	x ☐
y ☐						

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)
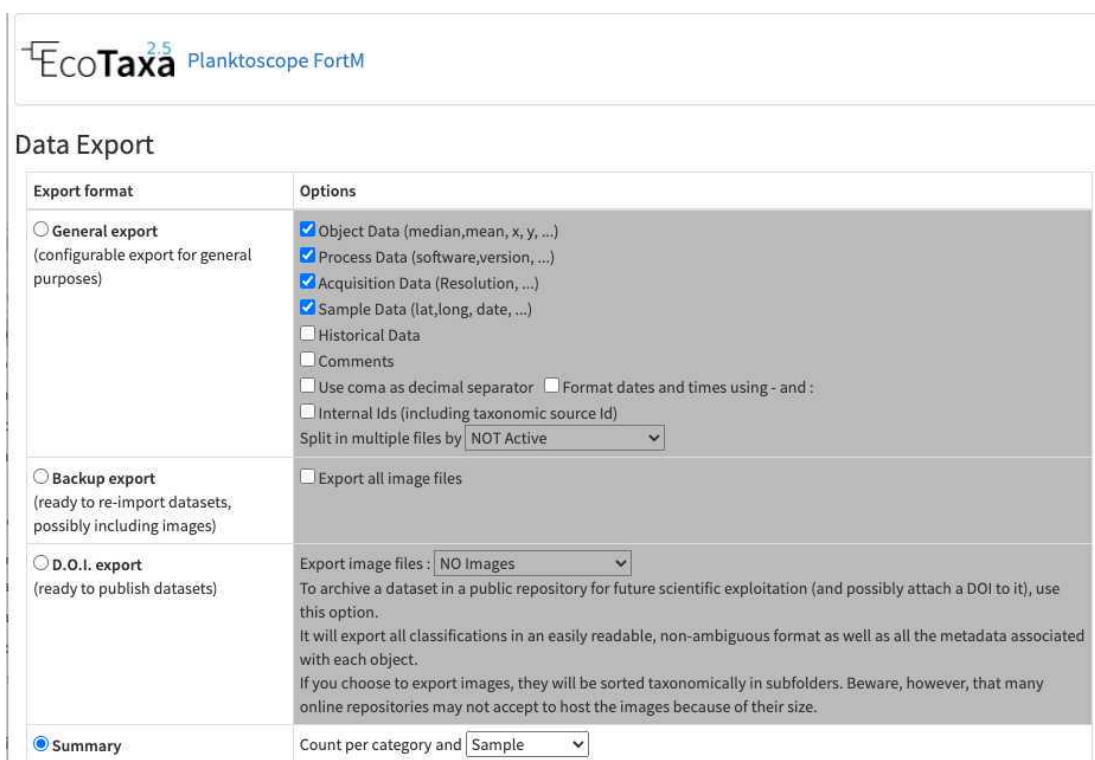

 example of images sorted by score of prediction

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)
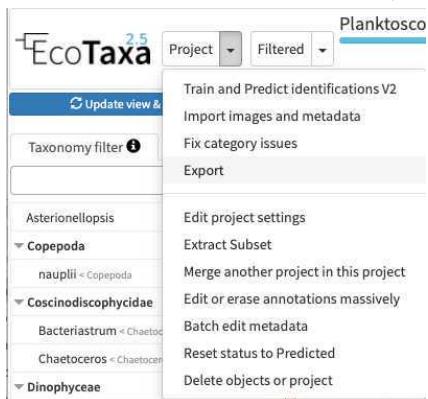
13   **Clean tubing and flowcell from inside**

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 ⬛ 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 (e.g. ⬛)

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 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 ⬛

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 ⬛

Troubleshooting

14 My flow cell is clogged with plankton, what to do?

Why this happens (preventive solutions):

- first this may happens if your sample does not have been "pre-filtered (we recommand pre-filtration to 200µm). Make sure to do this. ⬛ go to step #6.1

- It may aslo happens 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 ⮌ go to step #7.5

- it may also happens if you forget to agitate your sample using a bubble (⮌ go to step #3.11) or if you let 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). see ⮌ go to step #7.8

-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 luerlock 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.

External links

15   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)

https://forms.gle/qvh5jwuMvmyBKMQC7

Plankton Planet website

https://planktonplanet.org/

EcoTaxa tutorials:

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

https://www.youtube.com/watch?v=PSO6ZS765tk

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