

SHORT MANUAL TO SCAN and PROCESS SAMPLES

using the

ZOOSCAN

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Open access link in www.zooscan.com
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1. ZOOSCAN WORKING PROTOCOL

We do recommend doing daily backgrounds and nightly processing of the samples. The processing must be checked the next day before performing other tasks.

1.1 Morning: Check processing & Background scan.

1.1.1 Check processing of images using the dedicated tool in Zooprocess.

1.1.2 Perform background.

- Turn on the ZooSCAN and rinse the scan tray and the cover glass if necessary.
- Eliminate marks on the glass and frame and check from time to time if the glass of the ZooSCAN cover has not marks.
- Put some water to cover the tray (it avoids scratching the tray with the frame).
- Place the frame of your project (NARROW / LARGE).
- Fill with water until the step of the frame is covered.
- Do a background scan in the project you will work on (check that there is not dust on the OD (dark circle) position and that the tray and water are clean).
- DON'T FORGET TO PRESS THE TEMP GREEN LIGHT BUTTON before scanning (V1 and V2 only).

1.2 Day: SCAN samples

- Scan samples (while one sample is being scanned you can recondition the previous one or prepare the next one to gain time).
- COVER THE STEP OF THE FRAME WITH EXTRA WATER!
- PRESS THE TEMP GREEN LIGHT BUTTON if necessary (V1 & V2) before scanning!

1.3 End of the day

Launch a process of the images in batch mode, so all new images in the 'Raw' folder will be processed during the night.

1.4 When suitable

Perform prediction of Identifications (IDs) and validate the prediction.

1.5 If you do not have a Learning set to classify your organisms yet

You can dedicate some time to create your Learning set. We recommend not investing too much time on this task. Our strategy is using a small Learning set (~200 objects/group) to accelerate organism recognition, and then validating all the samples in order to have a robust dataset at the end. See the dedicated manual for these tasks.

2. ZOOSCAN DETAILED PROTOCOL FOR SCANNING

If it is the first time that you use your ZooSCAN, check that your ZooSCAN is on a bench without vibrations and that the instrument is horizontal (refer to Hydroptic manual for details).

2.1 First time working with ZooProcess

Refer to Zooprocess manual for installation.

2.2 Create a Project:

- Open Image J. Zooprocess starts (or click on the Z icon if ImageJ already started) and choose the option “create a new project” which is at the bottom of the project options’ list.
- Click OK.
- Choose the drive (we recommend to create your ZooSCAN projects in a drive root with nothing else stored (clean) (e.g., ZooSCAN root folder in C:, and ZooSCAN projects in D: drive).
- Select the scanning option: We recommend the largest image at 2400 dpi. (Narrow images at 4800dpi can be scanned if you run ZooSCAN V3 and Windows 7 pro 64 bits).

2.3 USER/ADVANCED modes

The default USER mode is activated when you create a project. It simplifies the daily work limiting possible manual errors.

You can switch to ADVANCED mode to get access to configuration tools and all options.

2.4 Fill sample metadata

Fill the SAMPLE metadata. This operation is no longer connected with the scanning of the samples. You can thus fill metadata for many samples, even if you will not scan them at the moment.

Consider carefully the naming convention of your samples!

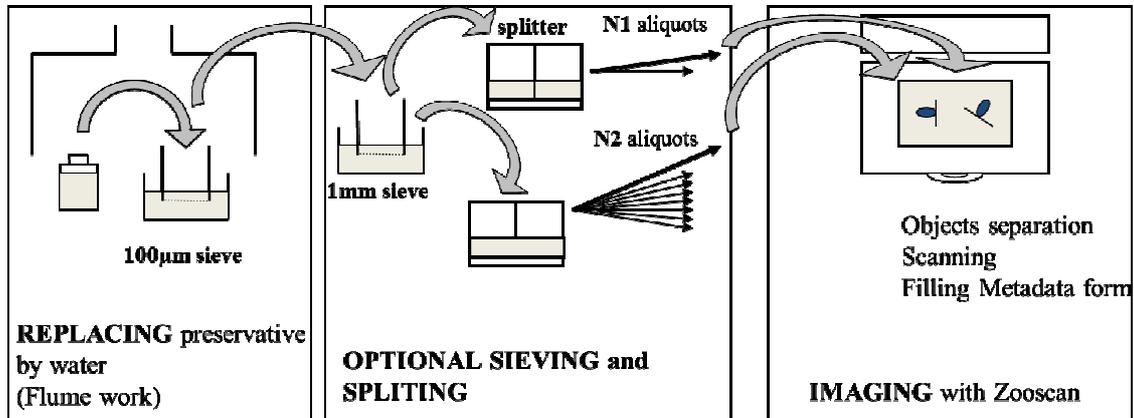
2.5 Sample preparation

We recommend storing liters of water at environmental temperature to work with your ZooSCAN. This will avoid condensation and bubbles related to temperature change between the tap pipes and the ZooSCAN room.

Treat the ZooSCAN gently and maintain it (e.g., always hold the tray when you lift it to remove the water or it could fall; also clean it and dry it at the end of the day). Avoid scratching the surface of the scanning tray by using only cactus sticks to separate organisms (these are provided with the ZooSCAN).

Check the performance of the ZooSCAN and make a blank by doing a background scan (2 replicates) every morning.

1. Turn on your ZooSCAN first thing in the morning (it needs to warm up). Leave it on the rest of the day if you are passing samples. Turn the ZooSCAN off during the night.
2. Prepare your sample. Procedure followed in Villefranche (for 200 and 330 μm sampling nets):



- a) Sieve your sample to take out the formal and sea water (you can keep it to recondition the sample afterwards). We then sieve it through a 1mm mesh and $200\mu\text{m}$ mesh to have 2 size fractions (this is to avoid missing the rare large organisms while splitting the sample to scan a subsample). Rinse the sample with tap water to properly eliminate the sea water. Place each fraction (subsample) in tap water.
- b) Now you have 2 size fractions: a large one ($>1\text{mm}$) ($d1$), and a small one ($200\mu\text{m}-1\text{mm}$) ($d2$). The tag ' $d1$ ' and ' $d2$ ' will be automatically added at the end of the scan name to distinguish them during the data processing.
- c) Take one of the fractions (e.g., $d2$) and split it until you have $\sim 1000-1500$ individuals (experience will give you the good eye for dilution to have a good scan: not too crowded, not poor).

2.6 Scanning samples

DO NOT FORGET TO DO A BACKGROUND (2 scans) every morning before a day of scanning.

1. Pour some water on the scanning tray until you have covered the glass.
2. Place the frame (the one defined in your ZooSCAN Project; preferably the large one). Important! Control that the frame is well placed on the recommended corner of the scanning tray (the area of scan is fixed to cover the frame from that corner).
3. Clean drops or marks on the frame.
4. Pour the sample and add tap water until all the perimeter of the frame's step is covered with water. You can accelerate the separation of organisms by pouring the sample homogeneously on the tray and by pouring the extra water on conglomerated areas of the tray to "dissolve" them.

3. Take 5-15 minutes to separate touching organisms. Move towards the center of the tray the organisms placed on the border of the frame or touching the frame (organisms touching the frame will be cut from the scan as the image is cropped on the borders of the frame). Also, if some organisms are floating, try to sink them by little pushes with the cactus spines (the size measurements of floating organisms are biased, and their image captions are blurred). If they do not sink and are very few, the best is to take them out of the image (e.g. placing them on the step of the frame). This step is critical to have good data quality. *Pay attention and try that none object is touching another. Nevertheless, some samples can be difficult to separate. Make a compromise between the time spent separating and the quality of the image. You can, after the process of the image, separate touching objects with the separation tool in zooprocess but you may lose details on the organisms and they may be truncated.*
4. Launch Zooprocess, select your project and click on “SCAN sample with ZooSCAN (for archive, no process)”. Then, follow the instructions on the zooprocess window.
5. Select the FRACTION name and fill the related metadata.
6. IMPORTANT for V1 and V2 versions! Do not forget to turn on the green light of the ZooSCAN before launching the scan in Vuescan. WAIT 30 seconds between the preview and pressing scan.

2.7 After scanning:

Your sample raw .tif image + log.txt file + meta.txt file will be created in your project folder:

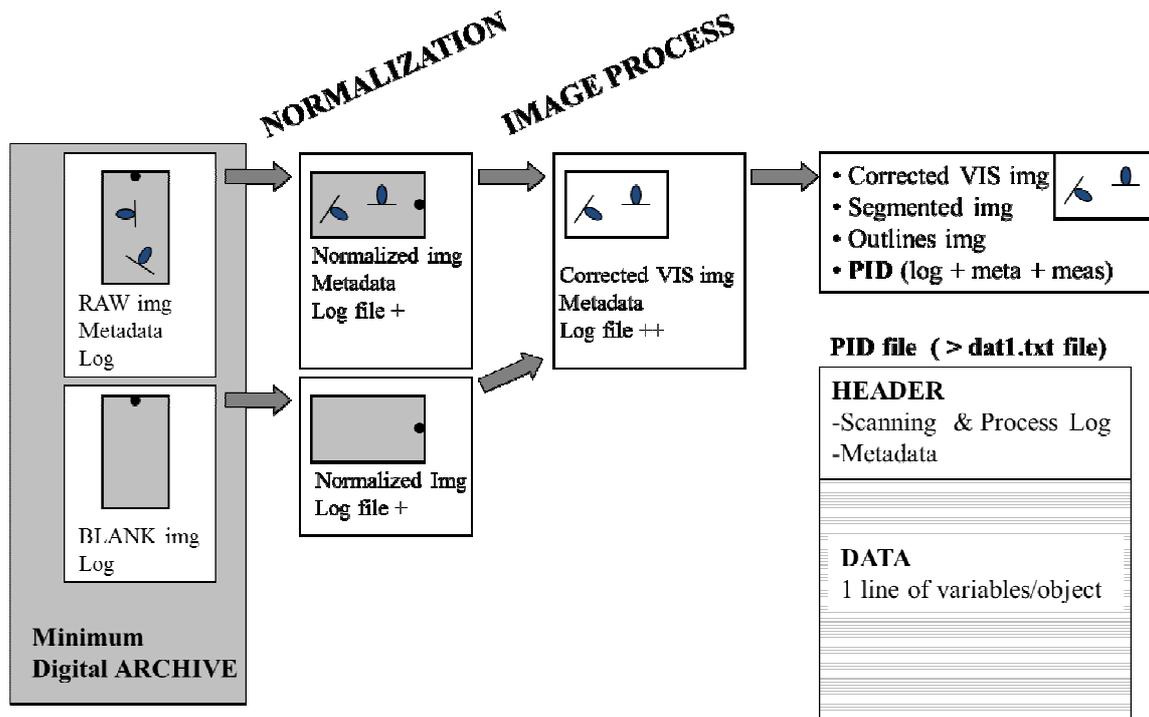
C:\Zooscan_project\Zooscan_scan_raw

The log file records information on the scanning method (parameters).

The meta file gives information on the sampling method (e.g., sampling site net dimensions, tow, volume) and on the sample preparation for the ZooSCAN (e.g., pre-filtering and splitting rate).

The image will be processed later with “Convert and process image in batch mode” (during the night).

3. PROCESSING SAMPLES



Note: it is recommended to launch the batch mode during the night.

1. If it is the first time that you process samples of your project, it may be useful to modify the size limits of the organisms that you want to analyze (keep same limits for all samples in one project for comparison!). You can change the ESD (Equivalent Spherical Diameter) thresholds of the objects to be considered in the scan (the default thresholds are 0.3 to 4 mm which encompass the mesozooplankton bulk).
2. To change the configuration of processing scans (not recommended except for ESD above), open ImageJ, click on Z as usual, select your project if you were in a different one the last time you used the program, (change mode to ADVANCED if necessary) and click on the option “Edit Configuration File”. All the options will be displayed in a box.
3. Start Zooprocess if necessary, click on the Z icon and select “CONVERT PROCESS IMAGES in batch mode”.
4. It is recommended to leave the configuration by default. You can extract the vignettes afterwards with the option “Extract vignettes for plankton identifier”; or much faster with the option “Extract vignettes in folder according to prediction” once the objects Ids have been predicted using Plankton Identifier.
5. At the end of the sample processing you will obtain a new subfolder named as the sample and placed in “Zooscan_scan_work\” of your project. In the subfolder you will have a new file, the .pid file. The .PID FILE is the one that you will use for your data analysis. It is a single file that concatenates the log.txt, the meta.txt, the processing functions applied and at the end the meas.txt which is the table containing all objects (rows) and their measurements (columns). The measurements that you might use to compute

size for data analysis are Area, Major (longest axis of the object) and Minor (minor axis of a perfect ellipse of the same area of the object measured). Other measurements correspond to variables of shape and texture for automatic recognition, and of position in the tray. Note that the PID file is automatically copied in the "PID_Results" folder at the end of the process.

4. CHECKING IMAGE QUALITY

Every morning, after a night of processing in batch, you select on Zooprocess "CHECK process by viewing segmented images". Select each image of the batch you performed. The opened image ("sample_msk1.gif") shows if the background was properly extracted from your image, (i.e., no saturated areas, with many dots). You can also check on this image the degree of aggregating organisms.

If you have doubts on the quality of your image, you may continue the quality check with the tools "view image with outlines" or also "view vignettes". These show you if more than one organism were considered as a single object by the system due to touching.

5. SEPARATING TOUCHING OBJECTS ON THE IMAGE

If you are not satisfied with the manual separation that you performed on the scanning tray (too many objects touching in the image), you can separate the touching organisms by drawing lines between them with the tool "Separation from B/W msk image".

NOTE: if you have done any separation since you processed your images, you must redo the PROCESS of samples to take benefit of the separation and get results for the resulting objects. In order to save computing time, select the option to reprocess automatically the images on which a recent separation has been made.