SHORT MANUAL TO SCAN and PROCESS SAMPLES

ZOOSCAN

Picheral, García-Comas, Desnos, Elineau, Romagnan

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J. Plankton Res. Gorsky et al. 32 (3): 285. : ZooSCAN methodological paper.

Open access link in www.zooscan.com
http://plankt.oxfordjournals.org/cgi/reprint/fbp124?ijkey=gJnvV9AjR20gITg&keytype=ref

Contenu

1. ZOOSCAN WORKING PROTOCOL

This working protocol might be adjusted by users according to their experience and needs in order to take advantage of the batch process modes provided by the application. Processes can be performed just after the scan if you use a powerful computer or during night or lunch time.

The typical analysis sequence is as follow:

- FILL IN sample metadata
- SCAN Background
- SCAN sample (one or several in a row)
- CONVERT and PROCESS samples (usually in batch mode)
- CHECK process
- SEPARATION of touching objects (Vandromme *et al.*, 2012)
    - On global image (best option), either B&W or in grey level
    - On vignettes
- PROCESS again if separation have been performed

2. CREATE A PROJECT

Please refer to the dedicated chapter in the Zooprocess manual. **A scanning project must always be created using the computer which is connected to the Zooscan.**

- Open Image J. Zooprocess starts (or click on the Z icon if ImageJ is already running) and choose the option “create a new project” which is at the bottom of the project options’ list.
- Click OK.
- Choose the drive
- Select the scanning option: We recommend choosing the "Large" image at 2400 dpi resolution. "Narrow" images at 4800dpi resolution can be scanned only if you run a ZooSCAN V3 with a Windows 7 pro (or 8) 64 bits computer.

3. USER/ADVANCED modes

The default USER mode is default when you create a project. It simplifies the daily work by limiting the options the user can access, and prevent most of possible manual errors.
You can switch to ADVANCED mode to get access to configuration tools and all options.

4. FILL IN SAMPLE metadata

This operation is no longer connected with the scanning of the samples. You can fill in metadata forms for several (many) samples, even if you intend to scan them in the future.

At this point you should have carefully considered the naming convention of your samples!

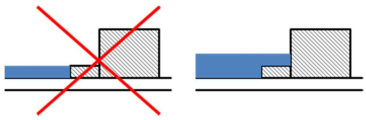
A sample table will be created by the application in the “meta” folder. This table lists all the samples and associated metadata you have filled in.

You can later edit and modify the metadata using the dedicated "**EDIT and MODIFY metadata**" tool in Zooprocess. This last tool will automatically modify all files containing the metadata.

5. SCAN a "background" image.

The background image is a blank image that will be used during the image analysis process. It should be scanned before the samples, in the same conditions as your samples images. It is recommended to scan a background image at the beginning of every scanning session.

- Turn on the ZooSCAN, clean and and rinse the scan tray and the cover glass using freshwater if necessary.
- Eliminate marks on the glass and the frame and check from time to time if the glass of the ZooSCAN cover has not marks.
- Pour some clean freshwater (stored at room temperature) to cover the tray (it prevents users from scratching the tray with the frame).
- Place the frame. At this point, be careful: the frame size (NARROW or LARGE) depends on your choices at step 2 "Create project".
- Fill the ZooSCAN tray with water until the step of the frame is covered.



- Scan a "background" image in the project you will work. (check that there is no dust on the OD (dark circle) position and that the tray and water are clean). Launch Zooprocess, select your project and click on "SCAN (CONVERT) background image".
- At this point, you must follow the instructions that pop up on your computer screen
- BIOTOM, V1 and V2 models: DON'T FORGET TO PRESS THE TEMP GREEN LIGHT BUTTON before scanning (V1 and V2 only) and wait recommended time (30 sec.) between preview scan and actual scan.
- V3 model: Check that the light rotating switch (left side of the Zooscan) is on UPPER position.

6. PREPARE SAMPLE.

IMPORTANT NOTE: Consider that FORMALIN is a carcinogen product. Apply all necessary safety procedure to remove all risks working in a flume to either remove formalin from the sample or scan the samples.

Working TIPS:

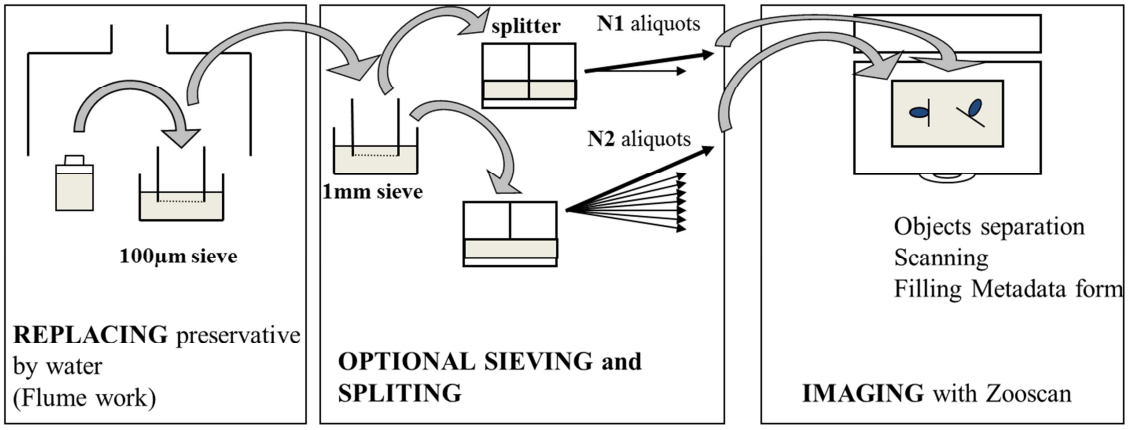
- We recommend to store few litres of freshwater (tap water is usually ok) at room temperature to work with your ZooSCAN. This will prevent condensation and bubbles to appear on the ZooSCAN tray and cover because of the temperature difference between the tap pipes and the ZooSCAN room.
- You can work either with freshwater or filtered seawater.
- Turn on your ZooSCAN first thing in the morning. Leave it on the rest of the day if you are scanning samples. Turn the ZooSCAN off after each scanning session.

Manipulate 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 scanning session). Avoid scratching the surface of the scanning tray by using only cactus sticks to separate organisms (these are provided with the ZooSCAN).

Prepare your sample in order to have suitable number of un-touching organisms for your final analysis. It may imply doing replicates and aliquots.

Example of preparation:

Protocol set up in Villefranche (for 200 and 330 µm sampling nets):



a) Sieve your sample to remove the preservative and sea water (you can keep it to re-do the sample afterwards). The sample is then sieved through a 1 mm mesh and 200 µm mesh to get 2 size fractions. This is done to prevent the underestimation of large and rare organisms.
b) Now you have 2 size fractions: a large one (>1mm) (d1), and a small one (200µm-1mm) (d2). The tag'd1' and 'd2' will be selected and 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 (subsampling) until you have ~1000-1500 individuals (only experience will make you able to get a suitable number of objects).

7. SCAN SAMPLE.

DO NOT FORGET TO DO A BACKGROUND (2 scans) every morning before any scanning session.

1. Pour water on the scanning tray until the glass is covered.

2. Place the frame (the one selected for your ZooSCAN Project; preferably the large one). Important! Control that the frame is well placed on the recommended and tagged corner of the scanning tray (the area of scan is set up to include the frame area and position, starting from that corner).

3. Clean water droplets 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 as for background.

5. Take 5-15 minutes to separate touching organisms.

   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. Do not add too much water. The step must be just covered.

   Place the larger individuals in the center of the tray because the image is cropped on the edges 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 to separating the objects from each other. Nevertheless, some samples can be difficult to separate. Make a compromise between the time spent separating and the quality of the image. After the process of the image, you can separate touching objects **on the final image** *with the separation tool in Zooprocess but you may lose details on the organisms and they may be truncated.*

6. Check for bubbles on the tray (cold water instead of room temperature water).

7. Check that there is no condensation on the glass of the ZooSCAN cover.

8. Launch Zooprocess, select your project and click on "SCAN sample with ZooSCAN (for archive, no process)".

9. Select the FRACTION name and fill the related metadata. Then, follow the instructions that pop up on the screen (Zooprocess window).

10. IMPORTANT for Biotom, 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 the actual scan.

11. IMPORTANT for V2 users: turn ON the UPPER light ONLY!

12. IMPORTANT for v3 users: select the UPPER light!

<u>FOR YOUR INFORMATION :</u>

- 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 subsampling ratio).

- The image will be processed later when using ”Convert and process image in batch mode” option in the Zooprocess main menu.

See Zooprocess manual for project architecture.

8. RECOVER SAMPLE.

1. Clean the recovering tray or jar to avoid contamination of the sample

2. Remove and rinse the transparent frame above the scanning tray to recover all specimens.

3. Lift gently and slowly the ZooSCAN tray to poor sample in the recovering tray or jar.

4. Rinse the tray with a squirt bottle to recover all organisms

5. Clean and DRY the scanning tray when you finish a scanning session.

9. PROCESSING SAMPLES: image analysis

Working TIPS:

- It is recommended to use the faster batch mode “CONVERT AND PROCESS” tool.
- It is also recommended to keep the default settings in all cases.

Procedure :

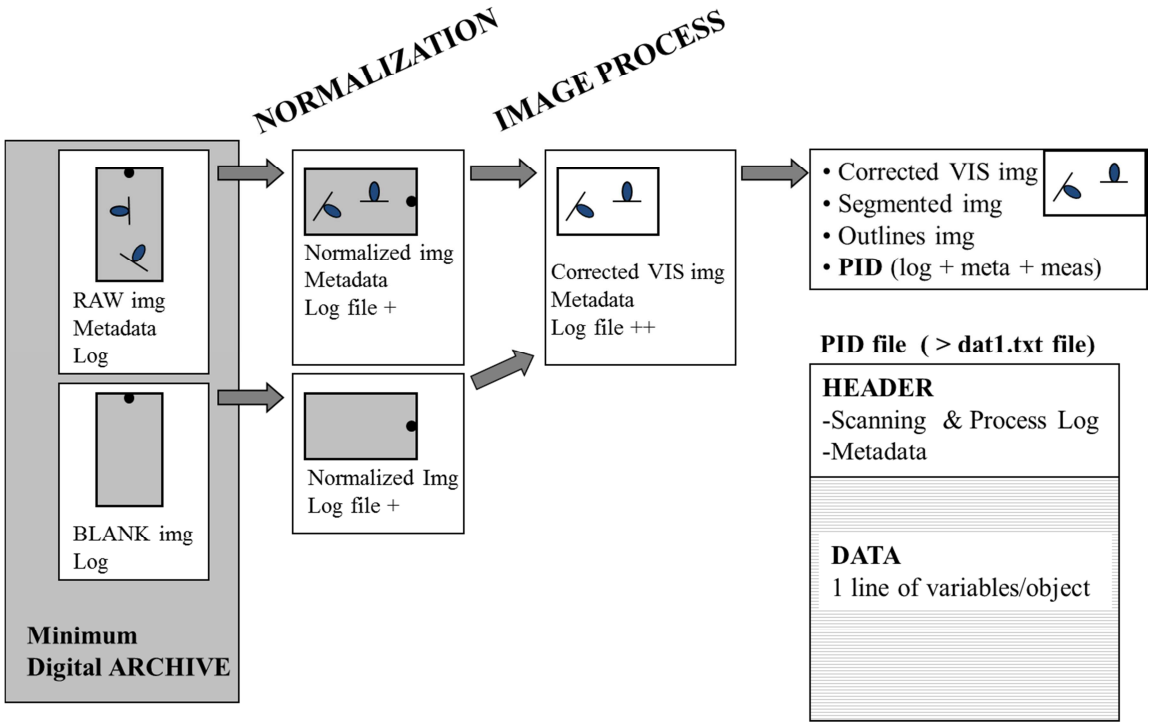
1. Start Zooprocess if necessary, click once on the Z icon and select “CONVERT & PROCESS images and organisms in batch mode”.

2. It is recommended to leave the configuration by default. Vignettes of scanned objects will be extracted by default (Zooprocess 7.18) in the sample folder along with the source image.

3. At the end of the sample processing a new subfolder named with the sample name is created in “ZooSCAN_scan_work\” of your project. In the subfolder a new file, the .pid file is created. The .PID FILE is used for data analysis. It is a single file that concatenates the log.txt, the meta.txt, the processing functions applied and meas.txt (table containing all objects (rows) and their measurements (columns)). The measurements that might be used to compute size for data analysis are Area, Major and Minor (major and minor axes of an ellipse that have the same area of the object measured). Other measurements correspond to variables of shape and texture used for

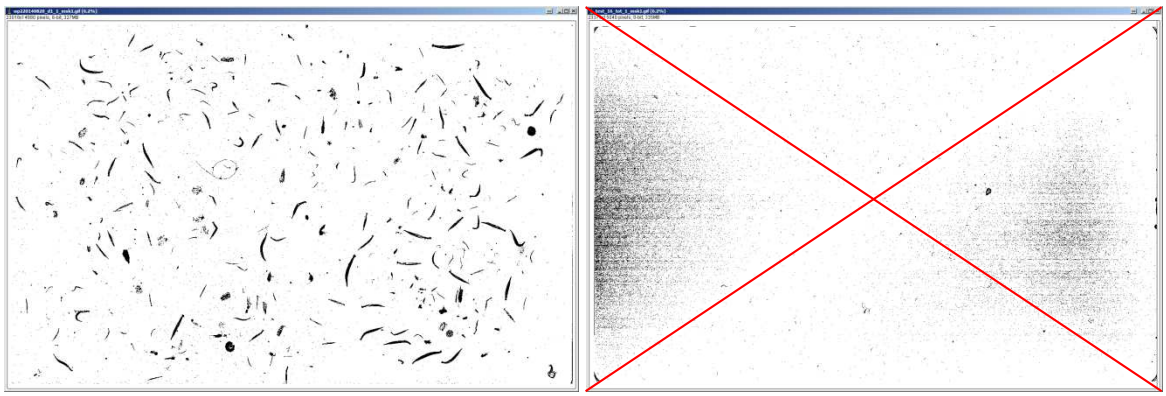
automatic recognition, and of position in the tray. Note that the PID file is automatically copied in the "PID_Results" folder at the end of the process.

4. Schematic image analysis steps



10. CHECKING IMAGE QUALITY

After any image process, you must check that the background was well subtracted from the sample image using the dedicated tool.
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.

11. CHECKING “Multiples” AND 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), if you notice that too many vignettes contains touching objects or that the B&W image shows too many touching objects, you should perform an additional separation on the image.

Use the tool “SEPARATION from global image (msk or vis)” to separate the touching organisms by drawing lines between them with the mouse.

Note that the separation from “vis” image requires more memory than the other.

12. RE PROCESS AFTER SEPARATION

If you have done any separation since you processed your images, you must redo the “CONVERT and PROCESS” of samples to take benefit of the separation and get results for the resulting objects.

In order to save computing time, select the last process option to reprocess automatically the images on which a recent separation has been made.