



# **ZOOSCAN**

# **MANUAL TO SCAN and PROCESS SAMPLES**

**Quantitative Imagery Plateform of Villefranche sur Mer (PIQv)** 

Picheral, Elineau

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J. Plankton Res. Gorsky et al. 32 (3): 285. : ZOOSCAN methodology paper.

Open access link in <a href="www.zooscan.com">www.zooscan.com</a>
<a href="http://plankt.oxfordjournals.org/cgi/reprint/fbp124?ijkey=gJnvV9AjR20glTg&keytype=ref">http://plankt.oxfordjournals.org/cgi/reprint/fbp124?ijkey=gJnvV9AjR20glTg&keytype=ref</a>

#### 1. IMPORTANT NOTE ON ZOOPROCESS VERSION

关于 Zooprocess 版本的重要提示

П	This protocol applies for the Zooprocess 7.27 version and above.
	本说明适用于 zooprocess 7.27 及以上版本
	DO UPGRADE Zooprocess as often as it is updated on the zooscan.com
	website: http://www.zooscan.obs-vlfr.fr/
	当 Zooprocess 在 zooscan.com 网站更新时,一定要经常升级:
	The PIQv will assist you only if you use the latest available update of Zooprocess
	只有你使用 Zooprocess 最新更新版本时 PIQv 才能帮助你。

# 2. ZOOSCAN WORKING PROTOCOL ZooSCAN 工作手册

This working protocol might be adjusted by users according to their experience and needs in order to take advantage of the tools provided by the application. We anyhow recommend to always keep the default settings offered by Zooprocess.

用户可以根据自己的经验和需要调整此工作手册,以便能充分利用该应用程序提供的工具。无论如何,我们建议始终保持 Zooprocess 提供的默认设置不变。

The typical analysis sequence is as follow: 典型的分析顺序如下:

-	1
	Fill in Sample metadata (as many sample as useful)
	填写样品元数据信息(可用样品越多越好)
	SCAN Background (on a daily base) 扫描背景(在每日基础上)
	SCAN sample (one or several in a raw) 扫描样品(在原始文件夹中一个或几个)
	CONVERT and PROCESS samples 转换和处理样品
	CHECK process 检测流程
	SEPARATION of touching objects 将彼此接触的对象分离开
	o On vignettes (best option) 在小图上(最好的选择)
	o On global image, either B&W or in grey level (old option)
	在球型图片上,在 B&W 或在灰度值水平上(以前的选择)
	PROCESS image again to include separation mask and get a better final dataset
	再次处理包含 separation mask 文件夹在内的图片,以便获得更好的终极数据库
	IMPORT in Ecotaxa to predict identification and manually validate or correct
	the prediction made by the application.
	导入到 Ecotaxa 来进行预测识别,并通过手动验证或更正由应用程序所做
	的预测。

# 3. CREATE A PROJECT 创建一个项目

A projet is a a folder where images and data of a cruise, survey or experiment will be saved. 一个项目就是一个用于保存航次、调查或实验图像和数据的文件夹。

• Open Image J. Zooprocess starts (or click on the Z icon if ImageJ is already running) and choose the option "CREATE NEW PROJECT" which is at the bottom of the project list.

打开 Image J, Zooprocess 自动开启(或者如果 Image J 已经运行,可以点击 z 键进行启动),选择项目列表下端的"CREATE NEW PROJECT" 选项。

- Choose the drive. 选择一个存储盘
- Select the scanning option: we recommend choosing the Large frame for images at 2400 dpi resolution. The Narrow frame for images at 4800 dpi resolution can be utilized only if you run a ZooScan V3 or V4 with a Windows 7 pro, 8 or 10 64

bits computer. It does not provide much better resolution and limit the scanning area and the number of organisms in the image.

选择扫描选项:我们建议选择大框,使获得的图像为 2400dpi 分辨率。只有当你在使用Windows 7 pro, 8 或 10 64 位的电脑系统,并使用 ZooScan V3 或 V4 版本进行运行的时候,才选择使图像达到 4800dpi 分辨率的窄框。



A folder will be created on the selected drive and all files from this project will later be saved in the specific subfolders.

一个文件夹会被创建在选定的存储盘内,并且这个项目的所有文件将随后被存储在特定的子文件夹中。

# 4. Fill in Sample metadata 填写样品信息

This operation is no longer connected with the scanning of the samples. You can fill in metadata in advance for several samples. You can even do it on the field and then move your project to the computer which is connected to the Zooscan.

这个操作与样品的扫描没有连接在一起,你可以提前为几个样品填写元数据信息,你 甚至可以在现场做,然后把你的项目转移到与 Zooscan 相连的电脑上。

At this point you should have carefully considered the naming convention of your samples! As it not possible to change the name of your sample afterward. And you should carefully check the format of several metadata such as Latitude and Longitude (see the metadata windows)!

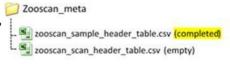
在这个节点上,你必须仔细考虑样品的命名!因为之后无法再更改样品的名称。并且你应该仔细地检查几个元数据信息的格式,例如纬度和经度(详见元数据信息窗口)。Do not use the "d1", "d2" or "tot" extensions in the sample names. These extends will automatically be added to the scan names at the time of the scan (see below).

不要在样品名称中使用"d1"、"d2"或"tot"等扩展名。这些扩展名将在扫描时自动地添加到扫描名称中(参见下文)。

#### An excel table named:

"zooscan\_sample\_header\_table.csv" will be created by the application in the "Zooscan\_meta" folder of your project. This table lists all the samples and associated metadata you have filled in.

一个名为"zooscan\_sample\_header\_table.csv"的 Excel 表格将被应用创建在你项目的"Zooscan\_meta" 文件夹中。这个表格列出了你已经填写的所有样品 及其相关元数据信息。



# 5. EDIT and MODIFY metadata 编辑和修改元数据信息

You can modify metadata (except the sample name) any time between all the steps clicking the option "EDIT and MODIFY metadata".

您可以在所有步骤之间的任何时间通过单击 "Edit / Modify metadata"选项来修改元数据信息(样品名称除外)。

You can EDIT metadata at any time to correct possible mistakes or complete information on the samples or the scans. Pay attention at these metadata. They will be essential for the later analysis of the results.

您可以在任何时候编辑元数据信息以纠正可能产生的错误,或完善样品和扫描中的信息。请注意这些元数据信息,它们对于后续的结果分析是非常重要的。

The tool corrects the metadata in the tables of the meta folder and inside all files related to the sample anywhere in the project.

该工具可以改正 meta 文件夹表格中的元数据信息,还有项目中所有与样品相关的文件。

# 6. SCAN (CONVERT) Background Image 扫描(转换)背景图片

The background image is a "blank" image that will be used during the image analysis process. It should be made before the samples are scanned and using the same parameters than for the scan of the samples. You have to scan a minimum of two background images (just follow the steps!) that will be combined in a unique "blank" image. You will do it at the beginning of every scanning session when you turn on your ZooScan, so usually every morning before the first sample.

背景图片是一个将在图像分析过程中使用的"空白的"图片。它应该在扫描样品步骤之前产生,并使用与样品扫描相同的参数。你必须扫描至少两个背景图片(遵循此步骤!),它们将结合成一个独特的"空白"图像。当你打开你的 ZooSCAN,每天早上扫描第一个样品之前,并开启每一个扫描进程的时候,你都必须做这一步骤。

The two background images will be processed into a unique background image and automatically saved in "Zooscan back" folder.

这两个背景图片将会被处理为一个唯一的背景图片,并自动保存在"Zooscan\_back"文件夹中。

- 1. Turn on the ZooScan (biotom, v1 and v2: power button on grey box; v3 or v4: power button behind the instrument), 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).
  - 打开 ZooSCAN 电源(biotom, v1 和 v2 版本:电源按钮在灰色盒子里; v3 和 v4 版本:电源按钮在仪器的后方),轻柔地操作和维护 ZooSCAN(例:当你 把样品池举起来倒掉水的时候,一定要一直扶着它,否则它可能会掉下来)。
- 2. Clean and rinse the scan tray and the cover glass using freshwater to remove the dirtiness.
  - 用淡水清洗和冲洗扫描池和盖板玻璃,以清除污垢。
- 3. Eliminate marks on the glass and the frame and check from time to time if the glass of the ZooScan cover has no mark.
  - 清除玻璃和样品框上的污垢,并不时检查 ZooScan 盖子上的玻璃是否没有污垢。
- 4. Pour some clean freshwater (stored at room temperature) to cover the tray. 倒一些干净的淡水(室温保存)盖住样品池。
- 5. Place the frame that was defined for your project (NARROW/LARGE). 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 borders). For biotom, v1 and v2 instruments there is a mark on the frame for his placement on the tray. Check the manuel delivered with the ZooScan.

放置你项目所需要的样品框(窄框/大框)。保证样品框被很好地放置在扫描池中建议和标记的角落(扫描面积被设置为包含框的边界)。对于 biotom, v1 和 v2 版本的仪器,框上有一个标记,说明其在样品池中的位置。检查 ZooSCAN 随机操作手册。

6. Fill the tray with freshwater until the edge of the frame is covered. Check that there is no dust or bubble below the OD (dark circle) position and that the tray and the water are clean: remove dirtiness and bubbles using a plastic pipette or a cactus stick provided with the instrument to avoid scratching the glass tray.

在样品池中加入淡水,直到框架的边缘被覆盖。检查在 OD (黑色圆圈)位置下方与样品池之间没有灰尘和气泡,并且水也是干净的:使用仪器自带的塑料吸管或仙人掌刺清除污垢和气泡,避免划伤玻璃样品池



Figure 1 : Filling the Zooscan tray

7. BIOTOM, V1 and V2 models: DON'T FORGET to turn on the light (green button or lever button) just before scanning. V3 and V4 models: Check that the light rotating switch (left side of the Zooscan) is on UPPER position.

BIOTOM, V1 和 V2 型号:开始扫描之前不要忘记打开灯(绿色按钮或杆按钮)。 V3 和 V4 型号: 检查灯的转动开关(Zooscan 左侧)处于上方位置。

8. Click on "SCAN (CONVERT) Background Image" tool. Follow the instructions that pop up on your computer screen. Wait the recommended time (30 sec.) between the preview, scan n °1 and scan n °2 (cf. the instructions window on your computer screen). Don't forget to press OK in the instructions window before the second scan.

点击"SCAN (CONVERT) Background Image"工具。按照电脑屏幕上弹出的提示操作。在预览,第一次扫描和第二次扫描(参考:电脑屏幕上的指令窗口)期间等待建议的时间(30s)。在第二次扫描之前不要忘记在指示窗口上按"OK"。

# SCAN and CONVERT Background (normalization)

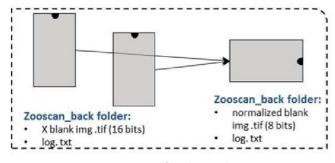




Figure 2 : Process of background images

# 7. Sample preparation and scan 样品准备和扫描

#### 7.1 Sample preparation 样品准备

Please do consider that the FORMALIN is a carcinogen and mutagen product. Apply all necessary safety procedure to remove all risks when you remove the formalin from the sample:

请务必考虑到福尔马林是一种致癌物和诱变剂产品,当你从样品中除去福尔马林时,请应用所有必要的安全程序以消除所有风险:

- □ work in a extractor hood 在通风橱中工作
- □ use gloves, glasses and lab coat 使用手套、眼镜和实验服

For safety reasons, we never poor directly the samples containing formalin on the Zooscan tray but we previously remove the seawater and formalin from the sample and use filtered seawater (or fresh water) instead.

出于安全原因,我们从不将含有福尔马林的样品直接导入到 ZooSCAN 的样品池中,而是从样品中去除海水和福尔马林,并使用过滤过的海水(或者淡水)替换。

We describe below the general procedure in use at the Plateforme d'Imagerie Quantitative de Villefranche sur Mer for 200 µm mesh size nets.

我们以 PIQv 使用 200 µm 网筛的过程为例描述以下的大体步骤。

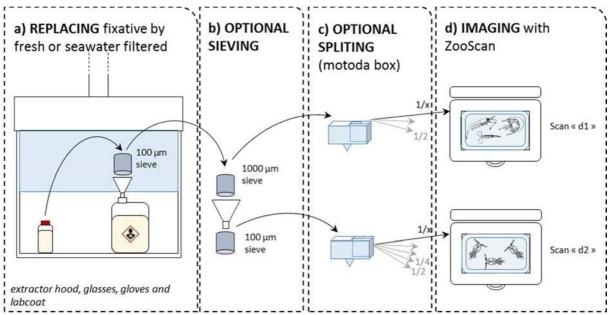


Figure 3: Procedure to prepare a sample collected using a WP2 net (200 µm): a) removal of the seawater and formalin, b) the sample is optionally sieved in two fractions, c) each of them is optionally splited in order to get convenient numbers of organisms and d) scan of both fractions ("d1" and "d2")

图3:使用WP2网(200 $\mu$ m)收集,进行样品准备的步骤: a)除去海水和福尔马林,b)样品被选择性的筛分成两个部分,c)每个进行随机的稀释,以便获得足够数量的生物体,d)扫描每一部分("d1"和"d2")

The sample is sieved under the extractor hood to remove the preservative and the sea water. You can keep it to re-fill the sample afterwards.

样品在通风橱中过筛,以除去防腐剂和海水,你可以之后再在样品中重新加上。

In order to prevent the underestimation of large and rare organisms and depending on the mesh size of the net utilized to collect the sample, the sample is then sieved through a 1000  $\mu m$  mesh and 100  $\mu m$  mesh to get 2 size fractions.

为了防止对大型稀有生物的低估,也取决于用于收集样品的网的孔径的大小,将样品通过 1000 µm 筛网和 100 µm 筛网,分别获得 2 个粒径分级。

The two fractions are then splited before scanning to obtain a proper number of organisms: a large one ( $>1000 \mu m$ ) and a small one ( $100 \mu m$ - $1000 \mu m$ ).

然后在扫描前将这两个部分稀释,以获得适当数量的生物体:大粒径部分(>1000 μm)和小粒径部分(100μm-1000μm)

#### NOTE: 注意:

☐ You can control that it is usefull to sieve the sample in two size fractions by comparing the splitting ratio necessary to obtain a good image or a correct quantity of organisms for the two fractions. **This ratio must be higher for the lower fraction.** 

对于这两个级别来说,你可以通过比较哪种稀释比率能获得更好的图片或者合适的组织数量,来控制把样品分成那两个粒径级别。这个比率必须比较低级别的部分更高。

□ By experience we scan 500-1000 organisms for the large fraction and 1000-1500 organisms for the lower fraction. These numbers can be adapted according to user experience and care. The number of touching organisms must be as limited as possible to avoid spending much time to later separate them from the images.

通过经验,我们对大粒径级别每次扫描 500-1000 个组织个体,小粒径级别每次扫描 1000-1500 个组织个体。这些数量可以根据使用者的经验和关注点进行调整。彼此接触的生物体的数量必须尽可能的少,以避免之后花太多的时间将它们在图片中进行分离。

You can decide to split the whole sample instead of using two size fractions (net mesh size  $> 500 \,\mu m$  for example).

你可以将整个样本进行稀释,而不用分成两种粒径级别(例如网筛尺寸>500 µm)。

□ In order to avoid mistakes during the splitting procedure using the Motoda box, we recommend to keep all successive remaining fractions in pre-labelled jars (1/2, 1/4, 1/8, 1/16....).

为了避免在使用 Motoda 分样器进行稀释过程中产生的错误,我们建议在所有后续剩余部分的瓶子上提前贴好标签(1/2, 1/4, 1/8, 1/16....)

# 7.2 STEP BY STEP SCANNING PROCEDURE 一步接一步的扫描过程

#### NOTES: 注意:

- □ The METADATA must be previously filled (see above) 元数据信息必须提前填好(参见上文)
- □ The BACKGROUND must be scanned (see above) 背景图像必须扫描(参见上文)
- 1. Pour some water (freshwater or filtered seawater) on the scanning tray until the glass is totally covered (don't cover the edges of the frame at this step as the addition of the sample will then cover the edge). In order to prevent condensation and bubbles to appear on the ZooScan tray (because of the differents temperatures between the working room and the water in the pipes), we recommend to anticipate your water usage and store few liters of water in the same room of the instrument.
  - 倒入一些水(淡水或者过滤过的海水)到扫面池中,直至玻璃被完全覆盖(在这一步不要覆盖到框架的边缘,因为添加的样本也会覆盖边缘)。为了防止在 ZooScan 样品池中出现冷凝和气泡(由于实验室中与水管中水的温度不同),我们建议你预估用水量,并在仪器所在的房间内储存少量的水。
- 2. Place the frame. The frame size (NARROW or LARGE) depends on your choices at step 2 "Create new project"). Check that the frame is well placed, touching the recommended and tagged corners of the scanning tray as for the scan of the background (see above).
  - 放置框架。框架的尺寸(窄框或者大框)取决于你在步骤 2 "Create new project"中进行的选择。查验框架被正确放置,要像背景扫描步骤一样,接触到样品池推荐和标记的角落(参见上文)。
- 3. Clean water droplets or marks on the frame and remove the dirtiness in you water with pipette and the cactus sticks.
  - 用吸管和仙人掌刺来清洁样品框上的水珠或水渍,清除水中的污物。

- 4. Pour the sample from the last fraction of the Motoda box 倒入 Motoda 分样器中最后一次稀释的样品。
- 5. Add water (if necessary) until all the edges of the frame are covered as for the background. If you pour too much water, the floating organisms will be out of focus. You thus have to struggle betwenn: enough water to cover the edges and an insuficiant level to avoid out of focus organisms.
  - 加水(如果必要的话)直到样品框的所有边缘被覆盖(如背景扫描步骤)。如果你倒了太多的水,漂浮的组织将会失去焦点。因此,你必须在两点间取得平衡:有足够的水覆盖边缘,又不至于过多使组织失去焦点。
- 6. Allow 5-15 minutes to separate touching organisms with the cactus sticks. No organisms on the edge and along the edge! Pay attention to the separation of the objects from each other. Make a compromise between the time spent for this task 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 (see below) in Zooprocess but you may loose details on the organisms and they may be truncated.
  - 花 5-15 分钟时间用仙人掌刺分离开彼此接触的组织。不要有组织在透明框的台阶上或者在台阶附近!在将对象彼此分离的时候要多加小心。花费在这项工作上的时间与图片的质量之间,你要做好取舍。在图片处理之后,你可以用Zooprocess 中的分离工具(见下图)分离最终图片上相互接触的物体,但是你可能会丢失生物的细节,它们可能会被截断。
    - ☐ You can accelerate the separation of organisms by pouring the sample homogeneously on the tray and by pouring the extra water on conglomerated organisms of the tray to "dissolve" them.
      - 你可以通过将样品均匀地倒在样品池上,以及在样品池中聚集生物较多的地方多加一些水来"分解"它们,从而加速样品的分离。
    - Place the larger individuals in the center of the tray because the image will be cropped on the edges of the frame. If some organisms are floating, try to sink them by little pushes with the cactus sticks (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 option is to move them apart of the image (e.g. placing them on the edge of the frame). This step is critical to have good data quality.

将较大的个体放在样品池的中心位置,因为如果在透明框的边缘,图像将会被裁剪。如果一些动物浮在水面上,试着用仙人掌棒轻压它们,使它们下沉(动物漂浮着的话,尺寸测量会有偏差,并且它们的图片会模糊)。如果它们不下沉的话并且又比较少,那最好的选择就是把它们从图片中分离出来(例如把它们放到样品框的台阶上)。这一步骤对于拥有良好的数据质量来说至关重要。

- 7. Check that there is no condensation on the glass of the ZooScan cover. 检查在 ZooSCAN 上盖的玻璃上没有冷凝水
- 8. Launch Zooprocess, select your project and click on the option: "SCAN sample with Zooscan (for archive, no process)".
  - 打开 Zooprocess,选择你的项目并点击"SCAN sample with Zooscan (for archive, no process)"选项。
- 9. Select the sample name. Then, follow the instructions that pop up on the screen (Zooprocess Instructions window). In Zooprocess, select the proper fraction name "d1", "d2", "tot" or user defined according to the fraction ( $100\mu m$ <"d2"< $1000\mu m$ , d1 >  $1000\mu m$ , "tot" if no sieving) when asked.

选择样品名称。然后,按照屏幕上弹出的指示操作(Zooprocess 指示窗口)。在 Zooprocess 中,选择合适的分级名称"d1"、"d2"、"tot",或者用户可以在被询问的时候根据粒径级别进行自定义(100 μm<"d2"<1000 μm, d1 > 1000 μm, 如果没有进行过筛就是"tot")。

10. Follow carefully the instructions in the Zooprocess windows to start the scan of the sample (one scan only). Do not forget to press OK in Zooprocess after pressing SCAN in Vuescan. Do not touch any key of the keyboard during the scan! Do not make any vibration on the Zooscan that may perturbate the water surface on the tray! Biotom, v1 and v2 Zooscan 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. v3: select the UPPER light!

小心地遵循 Zooprocess 窗口中的指示开始扫描样品(只扫描一次)。在 VueSCAN 中点击 "scan"之后不要忘记点击 Zooprocess 中的"OK"。在扫描 期间不要触碰键盘上的任何键!! 不要对 Zooscan 做任何可能干扰样品池水面稳定的振动! Biotom, v1 和 v2 Zooscan 版本:在 VueSCAN 上点击"scan"之前不要忘记打开 ZooSCAN 上的绿色灯。在预览和实际扫描之间等待 30s。v3 版本:选择上部灯光!

#### ADDITIONAL INFORMATION: 附加信息:

- Three files will be created in the "\_raw" folder of the project. The filename is composed of the sample name plus the fraction name (sample\_d1.\*) 三个文件将被创建在项目的"\_raw"文件夹下。 文件名字由样品名称加上分级名称(sample\_d1.\*)组成
- o TIF image (16 bits) will be processed later when using "CONVERT & PROCESS Images and organisms in batch mode" option in the Zooprocess main menu.

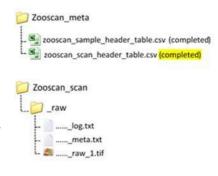
当使用 Zooprocess 主菜单的"CONVERT & PROCESS Images and organisms in batch mode" 选项时, TIF 图片(16位)将随后被处理

o 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). It summarizes the information contained in the sample and scan tables from the meta folder of the project.

META 文件夹提供了有关采样方法的信息(例如:采样点网型尺寸,拖拽方式,体积)还有为 ZooSCAN 所做的样品准备(例如前过滤和分样比率)。它总结了样品中包含的信息,还有从项目的 meta 文件夹中的扫描表格。

o LOG file records information on the scanning method (parameters).

LOG 文件记录了扫描方法的信息(参数)



- An additional scan\_header.csv file records all scanning information in the Zooscan\_meta folder
  - 一个额外的 scan\_header.csv 文件创建在 Zooscan\_meta 文件夹中,它记录了所有的扫描信息

# 8. RECOVER sample 回收样品

- 1. Remove and rinse the transparent frame with a squeeze bottle above the scanning tray to recover all specimens that may be stuck on it.
  - 取出样品框,并使用能挤压的洗瓶在样品池上方清洗样品框,以回收所有可能粘在其上的样品。
- 2. Remove the sample from the tray: lift the ZooScan gently and maintain it (e.g., always hold the tray when you lift it to remove the water or it could fall).
  - 从样品池里回收样品:轻轻地举起 ZooSCAN 并扶住(例:当你把它举起来倒出水时要一直扶着,否则它可能会掉落)。
- 3. Clean the tray with a squeeze bottle of freshwater or filtered seawater to avoid contamination between the differents samples.
  - 用装淡水或者过滤海水的洗瓶清洗样品池,避免不同样品之间互相污染。

Prepare another sample or clean and DRY the scanning tray using fresh water if you end a scanning session.

当你完成一次扫描后,你可以准备另一个样品,也可以使用干净的水清洗、擦干样品 池。

# 9. CONVERT & PROCESS Images and organisms in batch mode

# 批量模式转换 & 处理图像和样品

The image process can be performed just after the scan if you use a powerful computer or during night or lunch time.

如果你使用一台配置较高的的电脑,或是在晚上或午饭时间,扫描后可以立即进行图片处理。

It is very important to process the scanned images as soon as possible after their acquisition or at least every day. The process permits to rapidly check (see below) that the background and the samples have been correctly acquired.

在获取图像之后尽快将扫描到的图像进行处理是非常重要的,或者至少每天处理一次。 这个过程可以让我们快速地检验(参见下文)我们的背景和样品已经被正确的获取到 了。

- 1. Start Zooprocess if necessary, click once on the Z icon and select the tool "CONVERT & PROCESS Images and organisms in batch mode" and then "Convert AND Process Image AND particles (images in RAW folder)". It is recommended to keep the default settings for the process.
  - 如果有需要的话可以打开 Zooprocess,点击 "Z"键,并且选择"CONVERT & PROCESS Images and organisms in batch mode"工具,然后选择"Convert AND Process Image AND particles (images in RAW folder)"。对于这个过程来说,推荐保持系统的默认设置。
- 2. At the end of the process, a Zooprocess window appears with a "NORMAL END..." message.
  - 在处理完之后,一个 Zooprocess 窗口会提示"NORMAL END..."信息。

### FOR YOUR INFORMATION: 供参考的信息:

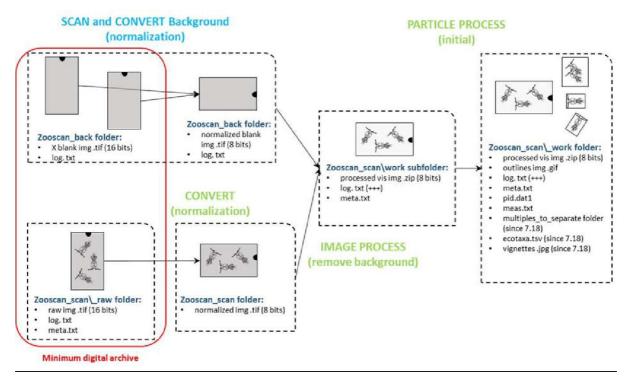


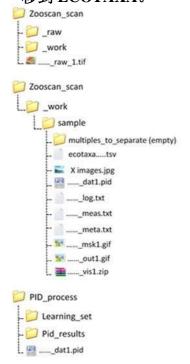
Figure 4: Description of the standard initial background and image processes. The operations CONVERT, IMAGE PROCESS and PARTICLE PROCESS are all included in the "Convert AND Process Image AND particles (images in RAW folder)" tool. 图 4: 对标准化初始背景和图像处理的描述。转换,图像处理和小图处理操作全包含在"Convert AND Process Image AND particles (images in RAW folder)"工具中

● The resulting files are saved in the \_work and Pid\_results folders. 结果文件储存于 work 和 Pid\_results 文件夹中。

The .pid file is a single file that concatenates the log.txt, the meta.txt, the processing functions applied and the meas.txt (table containing all objects (rows) and their measurements (columns)). The measurements that might be utilized to compute the size for the 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 of your project.

- .pid 文件是连接 log.txt 和 meta.txt、处理功能应用和 meas.txt(包含所有对象(行)和它们的测量值(列)的表格)的单个文件。可以用来计算数据、分析尺寸的测量值是面积、主轴和副轴(具有相同测量对象面积的椭圆的主轴和副轴)。其他测量值与用于自动识别的形状和纹理变量相对应,还有其在样品池中的位置。注意: PID 文件会被自动拷贝到你项目的 PID Results 文件夹中。
- Since the Zooprocess 7.22 version: vignettes of scanned objects are extracted by default and an "ecotaxa\_\*.tsv" file is created. They are both imported into the ECOTAXA (<a href="http://ecotaxa.obs-vlfr.fr/">http://ecotaxa.obs-vlfr.fr/</a>) application to predict and classify the organisms. The TSV file contains all data and all useful metadata from the PID file (see below).
  - 从 Zooprocess 7.22 版本开始: 扫描对象的小图被默认提取,并且一个 "ecotaxa\_\*.tsv" 文 件 会 被 创 建 。 它 们 都 被 导 入 到 Ecotaxa (http://ecotaxa.obs-vlfr.fr/)应用中进行样品的预测和分类。TSV 文件包含着来源于 PID 文件中的所有的数据和所有有用的元数据信息
- The resulting files remain compatible with the old Plankton Identifier and XnView tools but we DO NOT recommend to continue to use them. You can contact us in order to get assistance to move your already classified samples/projects into ECOTAXA.

生成的文件仍然与旧的 Plankton Identifier 和 XnView 工具兼容,但我们不建议继续使用它们。您可以联系我们,以便获得帮助,将您已经分类的样品/项目转移到 ECOTAXA。



#### 10. CHECK process by viewing segmented images

# 通过查看分割的图像来检查处理过程

After any image process, you must check that the background was well subtracted from the sample image using the dedicated checking tool: "CHECK process by viewing segmented images". 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 aggregated organisms.

任何图片处理之后,你都必须使用专用的检查工具: "CHECK process by viewing segmented images" 检查背景是否从样品图像中很好地去除。打开的图片 ("sample\_msk1.gif")显示了是否从图像中正确去除了背景(例:不饱和区域,有许多杂点)。你也可以通过这张照片检查生物体的聚集程度。

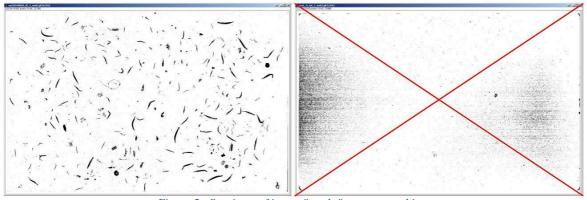


Figure 5: Specimen of image "masks": correct and incorrect **图5: 样品图片: 正确和错误示范** 

Some basic reasons for bad quality images can be:

关于图片质量较差的可能原因:

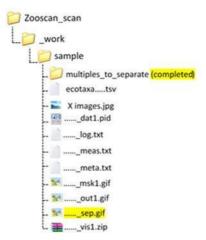
- ➤ Too many organisms 组织样品过多
- ➤ Too many very large organisms or badly positionned 大型样品太多或者放置位置错误
- ▶ Bad background (dirty, condensation, insufisant quantity of water) => all samples of the same day (processed using the same background) present similar defaults 背景很差(脏、冷凝、水量不够)=> 同一天的所有样品(使用相同的背景进行处理)都存在相同的默认值。
- ➤ Bad sample (dirty, condensation, insufisant quantity of water) 样品质量差(脏、冷凝、水量不够)
- ➤ Insufficient delay between the Preview of the sample and its scan 样品的预览和扫描之间的时间差不足。

# 11. CHECKING "Multiples" and SEPARATION using selected vignettes of multiples 检查"多个对象"并对选定的含有多个对象的小图进行分离

If you are not satisfied with the manual separation that you performed on the scanning tray (too many objects touching in the image) and you notice that too many vignettes contains touching objects, you should perform an additional separation on the vignettes.

如果你对在扫描池中进行的手动分离过程不满意(图像中互相接触的对象太多),并且你注意到有太多的小图中包含互相接触的对象,那么你应该对小图进行额外的分离。

- 1. Use XnView or any image browser application to check the sample folder (sub folder of the \_work folder) for vignettes containing touching objects and move (cut or copy) them into the "multiples to separate" subfolder.
  - 使用 XnView 或其他的图像浏览器来查看样品文件夹(\_work 文件夹的子文件夹)中有互相接触对象的小图,并将它们移动(剪切或复制)到"multiples to separate"子文件夹中。
- 2. Use the tool "SEPARATION using selected vignettes of multiples" to separate the touching organisms by drawing lines between them. The latest version of the tool (Zooprocess 7.25) permits to draw many lines on a single vignette in a batch.
  - 使用"SEPARATION using selected vignettes of multiples"工具并通过画线的方式,来对互相接触的个体进行分离。该工具的最近版本(Zooprocess 7.25)允许在同一张小图上多次画线。



# 12. RE-PROCESS after separation 分离之后再处理

You must redo the "CONVERT & PROCESS Images and organisms in batch mode" and select "Process again particles from processed images to include SEPARATION MASK (images in WORK sub-folders)" of samples to take benefit of the separation and get results for the resulting objects. At the end of the sample processing a zooprocess window appears with "NORMAL END".

你必须重新进行"CONVERT & PROCESS Images and organisms in batch mode"步骤,并选择"Process again particles from processed images to include SEPARATION MASK (images in WORK sub-folders)"选项,以利于样品的分离并获得最终的结果。在样品处理的最后,Zooprocess 软件会弹出"NORMAL END"窗口。

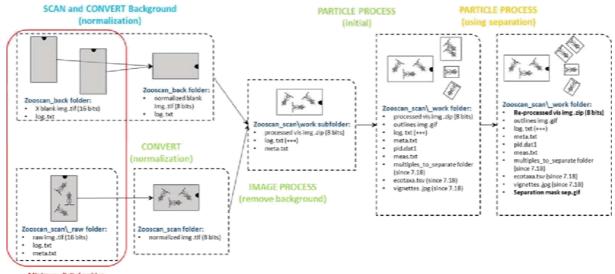
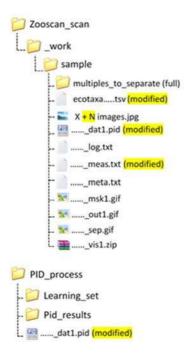


Figure 6: Standard processes plus additional separation of touching objects **图6:** 标准处理程序加互相接触对象额外的分离



# 13. USER/ADVANCED modes for a project

# 一个项目下的 USER/ADVANCED 模式

The USER mode is set as default when you create a project. It simplifies the daily work limiting the options the user can access, and preventing most of possible manual errors.

After entering a project, you can switch to the ADVANCED mode to get access to the configuration tools and all other options selecting the "SWITCH Mode" tool, wich is at the bottom of the options list.

创建项目时将用户模式设置为了默认模式。它可以简化日常工作,并限制了用户可以访问的选项,还能防止大多数可能发生的手动错误。进入项目后,你可以通过选择位于选项列表最下方的"SWITCH Mode"工具切换到高级模式,以便访问配置工具和所有其他选项。