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NOT TO BE CITED. A manual by Jose A. Fernandes based on manuals written by Philippe Grosjean and Kevin Denis: <http://www.sciviews.org/zooimage/>
Note: It is a specific manual that only uses some of the functionality of ZooImage. For more information consult ZooImage manuals and website.

12. Zooplankton Tutorial using an Spread Sheet

12.2.1. Introduction

In this tutorial, an example of 10 zooplankton images is used to show you the process of image treatment done by the software. These colour images are acquired from scanner colour connected to a computer. They proceed from specialists of Azti Tecnalia, and they were selected from a series of sample realized across the Bay of Biscay. This data set, provided with ZooImage, allows you to test the software. Because all photographs are not succeeded, the data set presents a gap in the name of images (the seventh photography is missed). The software can take this sinning in consideration during the importation of images (see below). For all this process a Spread Sheet is going to be used for introducing metadata to make more easy and comfortable the process to technicians. In this example a excel file is used, but it can be used any other kind of Spread Sheet.

12.2.2. Resumed advices

1. Give names like p-0001.jpg to your samples instead of p-1.jpg. There is some automatic renamers out there. Be carefull with missing stations.
2. Remove from spread sheet stations where there is no image.
3. Use the current date format (yyyy-mm-dd): 2005-12-22.
4. Use “.” notation for decimal number.
5. Do not change order or names of columns in the excel file.
6. Random forest can not have empty folders in the training set, neither only one item. It should hava at least two.

12.2.2. Scanning images

An advice: Give names like p-0001.jpg to your samples instead of p-1.jpg.

12.2.1. Getting ready with the spread sheet

Use the spread sheet of the example has a template, feel free to change or adapt it to your necessities. For your consideration:



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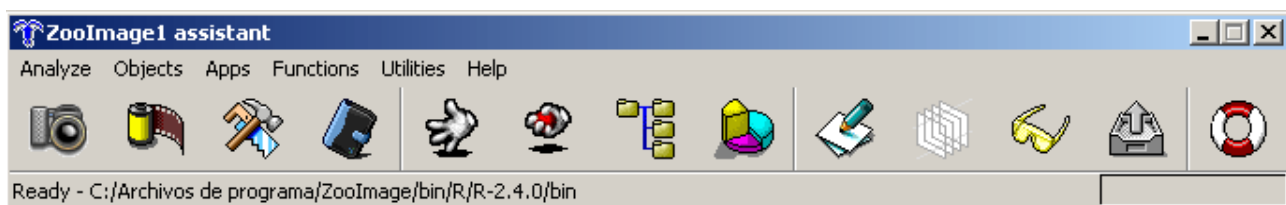
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1. In the spread sheet there will be fields that have values directly; you just time the correct one for your samples.
2. Or the field can be a calculated one, this is to save time; most of times, instead of modifying that field, you should modify the fields that is the source for the calculated value.
3. The order of columns should not be altered, you can add more columns at the end, but do not change the existing ones, or ZooImage could not work properly and you will not be able to know why (working on improving this).
4. The name of columns should not be changed or ZooImage will not work properly. The names are case sensitive.
5. Be careful with dates format, it have to be yyyy-mm-dd. But be careful sometimes excel shows that format, but if you go to the field, you can see in the top that is storing it with another representation.
6. Preferably use “.” notation, instead of “,” for decimal amounts. We are working in doing this indifferent.
7. SubPart should have at least four digits of decimal precision.
8. VolIni should have at least three digits of decimal precision

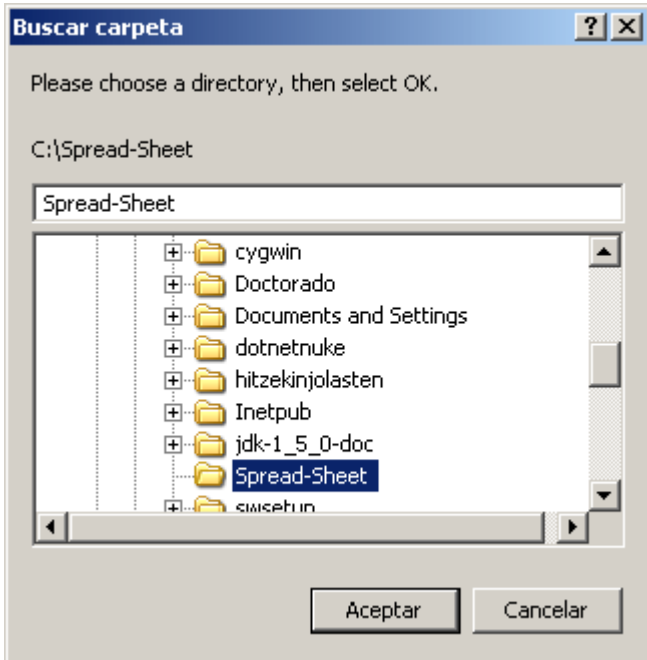
12.2.2. Import images

Prepare an empty directory on your hard disk (let’s say, C:\Spread-Sheet, but you can freely choose another partition or directory name).

Change the active directory there, using the Options → Change active dir... menu and select that directory.



Select Options → Change active dir...in the menu



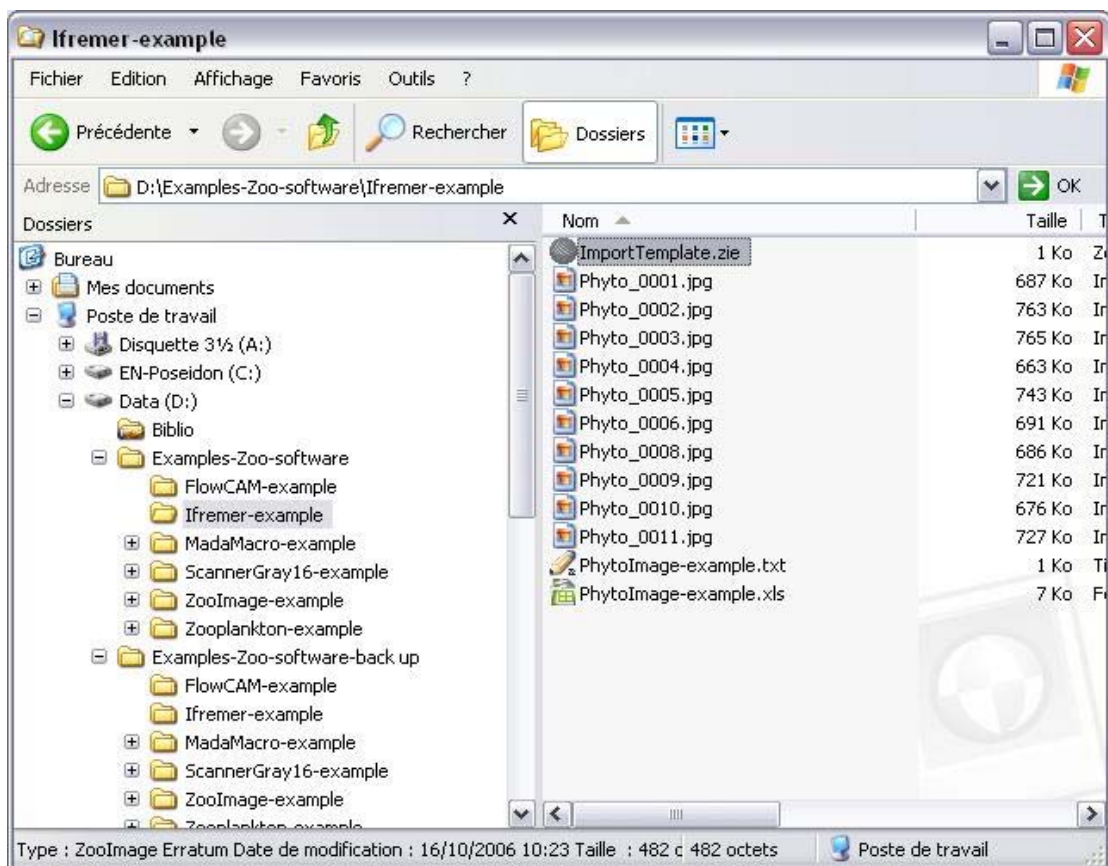
Select the directory you just created



Your directory is now the active one, as you can see in the assistant status bar.

Copy the “SpreadSheet-example” in this directory that can be downloaded from the ZooImage WebSite.

You should have something like this: FALTA CAMBIAR IMAGEN



Exporting the table from Microsoft Excel

Open ZooImage-template.xls into Microsoft Excel. This file is already complete for our ten example images, but you can then see how it should look. It can be used as a guide to put your own data.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Sample	Image	Cam	Type	Stn	Date	Fecha	LAT_PV	LONG_PV	T°S	Vollni	vol probeta	vol alicuota	SubPart
2	BIO. 1998-5-18. P0001+A	0001	BIO	A	p-1	1998-05-18	18-5-98	432830	25107	16.6	8.1207	216	6	0.02777777
3	BIO. 1998-5-18. P0002+A	0002	BIO	A	p-2	1998-05-18	18-5-98	433151	25167	17.0	9.0731	232	6	0.0258620E
4	BIO. 1998-5-18. P0003+A	0003	BIO	A	p-3	1998-05-18	18-5-98	433448	25168	16.2	8.3525	234	6	0.02564102
5	BIO. 1998-5-18. P0004+A	0004	BIO	A	p-4	1998-05-18	18-5-98	433751	25177	15.6	9.0250	202	6	0.02970297
6	BIO. 1998-5-18. P0005+A	0005	BIO	A	p-5	1998-05-18	18-5-98	434055	25185	15.6	8.9633	206	6	0.02912621
7	BIO. 1998-5-18. P0006+A	0006	BIO	A	p-6	1998-05-18	18-5-98	434352	25191	16.0	9.1961	222	6	0.02702702
8	BIO. 1998-5-18. P0007+A	0007	BIO	A	p-7	1998-05-18	18-5-98	434651	25195	15.5	9.0371	222	6	0.02702702
9	BIO. 1998-5-18. P0008+A	0008	BIO	A	p-8	1998-05-18	18-5-98	434951	25201	15.7	9.5010	220	6	0.02727272
10	BIO. 1998-5-18. P0009+A	0009	BIO	A	p-9	1998-05-18	18-5-98	435258	25201	15.3	9.9051	224	6	0.02678571
11	BIO. 1998-5-18. P0010+A	0010	BIO	A	p-10	1998-05-18	18-5-98	435549	25205	15.1	9.3791	220	6	0.02727272
12														

In our example, we have ten samples and ten images, one image for each sample (in a real-world application, you will have, of course, much more samples and many more images per sample



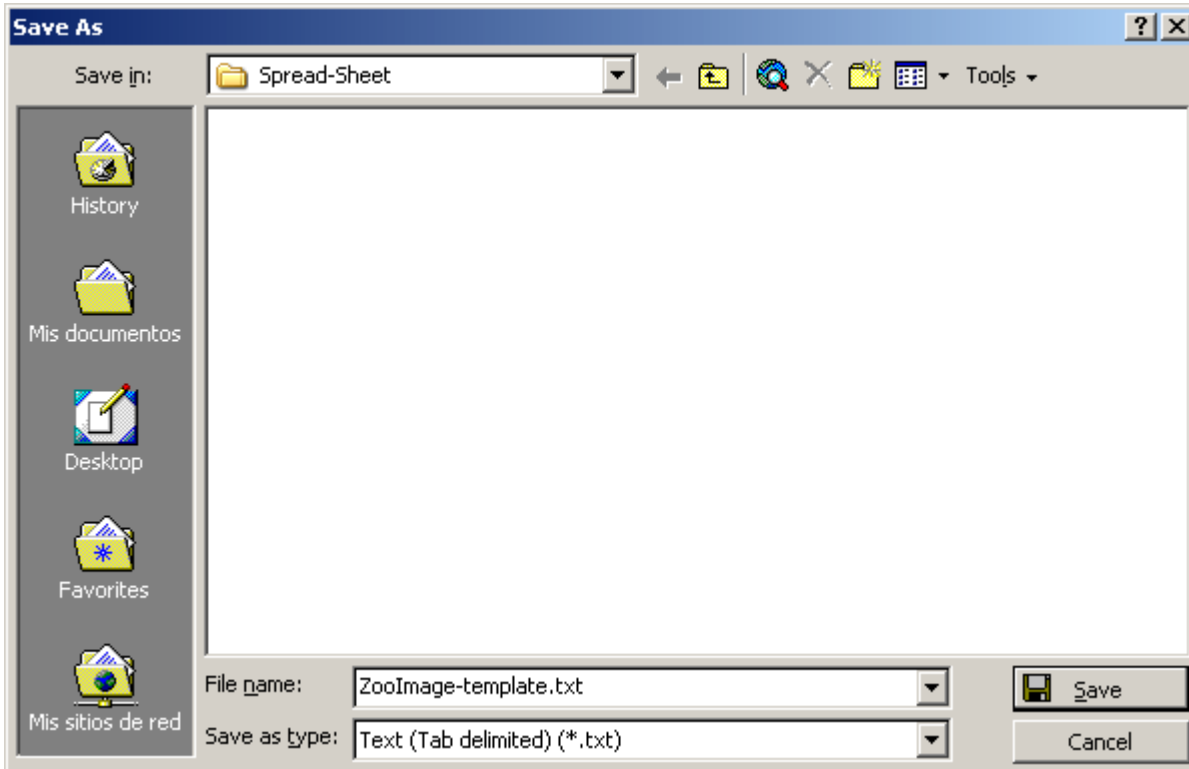
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to process... but the mechanism remains essentially the same). You must create a table with the first column having the following headers: ‘Sample’, ‘Image’, ‘Campain’, ‘Type’, ‘Stn’ and ‘Date’. This fields must exist with that name and in that order. VollIni and SubPart must exist too, with that name, but can be in any order. ‘Sample’ is the name of the different samples. ‘Image’ is the list of sequences of the images to use for each sample. All the other columns are calibrations and quantitative determinations of the. **You can add new column like here with “LAT_PV”, this new column will be automatically considered by the software during importation.** BUT NEVER CHANGE ORDER OR NAME OF FIELDS IN BLUE. AND NEVER CHANGE NAME OF FIELDS IN ORANGE. In addition, this fields always need to have data.

Once this table is compiled, save it in plain text format. Select File → save as... menu entry.

	E	F	G	H	I	J	K	L	M	N
	Stn	Date	Fecha	LAT_PV	LONG_PV	T°S	VolIni	vol probeta	vol alicuota	SubPart
2	p-1	1998-05-18	18-5-98	432850	25107	16.6	8.1207	216	6	0.02777777
3	p-2	1998-05-18	18-5-98	433151	25167	17.0	9.0731	232	6	0.0258620E
4	p-3	1998-05-18	18-5-98	433448	25168	16.2	8.3525	234	6	0.02564102
5	p-4	1998-05-18	18-5-98	433751	25177	15.6	9.0250	202	6	0.0297029
6	p-5	1998-05-18	18-5-98	434055	25185	15.6	8.9633	206	6	0.02912621
7	p-6	1998-05-18	18-5-98	434352	25191	16.0	9.1961	222	6	0.02702702
8	p-7	1998-05-18	18-5-98	434651	25195	15.5	9.0371	222	6	0.02702702
9	p-8	1998-05-18	18-5-98	434951	25201	15.7	9.5010	220	6	0.02727272
10	p-9	1998-05-18	18-5-98	435258	25201	15.3	9.9051	224	6	0.02678571
11	p-10	1998-05-18	18-5-98	435549	25205	15.1	9.3791	220	6	0.02727272

In the Save as dialog box, change the type to ‘Text (Tab delimited) (*.txt)’. **MAKE SURE THERE IS NO EMPTY LINES AT THE END OF THE FILE.**



A couple of warning messages will be displayed, but you can ignore them (just click ‘OK’ and ‘Yes’, respectively).

At the end, you should have a file ZooImage-template.txt file created in the same directory as your original ZooImage-template.xls file. **Close Excel** (this is very important, otherwise, any other program cannot access the file you just created!). **It is possible that you have to replace the existing .txt file by the new.**

Proceeding the table to import images and built the metadata files: first importation method

Whether you created your table with Microsoft Excel or OpenOffice Calc, you should end up with something similar to this, when opened in a plain text editor.

ZoolImage-template.txt

Sample	Image	Campaign	Type	Stn	Date	Fecha	LAT_PV	LONG_PV	T ^a
BIO.1998-5-18.P0001+A		0001	BIO	A	p-1	1998-05-18			18
BIO.1998-5-18.P0002+A		0002	BIO	A	p-2	1998-05-18			18
BIO.1998-5-18.P0003+A		0003	BIO	A	p-3	1998-05-18			18
BIO.1998-5-18.P0004+A		0004	BIO	A	p-4	1998-05-18			18
BIO.1998-5-18.P0005+A		0005	BIO	A	p-5	1998-05-18			18
BIO.1998-5-18.P0006+A		0006	BIO	A	p-6	1998-05-18			18
BIO.1998-5-18.P0007+A		0007	BIO	A	p-7	1998-05-18			18
BIO.1998-5-18.P0008+A		0008	BIO	A	p-8	1998-05-18			18
BIO.1998-5-18.P0009+A		0009	BIO	A	p-9	1998-05-18			18
BIO.1998-5-18.P0010+A		0010	BIO	A	p-10	1998-05-18			18

This file, together with the ImportTemplate.zie file, located in the same directory, will tell to ZooImage how to compile instructions to import your images. We present just below the content of the ImportTemplate.zie file for our example images.

ImportTemplate.zie has various parameters: [Table to do]

[Image]: Various parameters of the image acquisition system (in our case, a scanner).

[Import]: *FilenamePattern=Phyto_<4>.jpg* : Name of the file with 4 descriptive numbers.

FractionPattern= : Fraction digitized of the sample.

SubsamplePattern=????????????????

Convert= Software used to convert the raw image into an other readable format.

FileExt= Extension name of the file format.

FileConv= Convention parameters to use to transform the other format into a jpg format.

Return= Parameters of the program to convert the raw data in an other format.

FileExt2=jpg : Final format.

Nmin=5 : Minimum number of picture for each sample (default value).

Nmax=5 : Maximum number of picture for each sample (default value).

[Fraction]: *Code=A or B* :Size fraction of the sample (i.e : A= -50 μm, B= +50 μm).



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[Calibration]: *PixelSize* : Size of the pixel.

PixelUnit : μm in our case.

[Subsample]: *SubPart=NA* :Subsampling part.

SubMethod= Subsampling method.

CellPart=????

Replicates=1 : Number of replicate or name of replicat???

VolIni= number of cubic meter filtered by the net.

VolPrec=Precision of the net sample.

```
ImportTemplate.zie
File Edit Search Project View Format Column Macro Advanced Window Help
ZI1
[Image]
Author= AZTI - Tecnalia - Unidad de Investigación Marina
ImageType=trans 8bits color 600dpi
Hardware=hp Scanjet 8200
Software=Digital Imaging

[Import]
FilenamePattern=p-<4>.jpg
FractionPattern=
SubsamplePattern=
Convert=
Return=
FileExt=
FileConv=
FileExt2=jpg
Nmin=5
Nmax=5

[Fraction]
Code=A
Min=-1
Max=-1

[Subsample]
SubPart=
SubMethod=volumetry
CellPart=1.00
Replicates=1
VolIni=
VolPrec=
CellPart=1
```

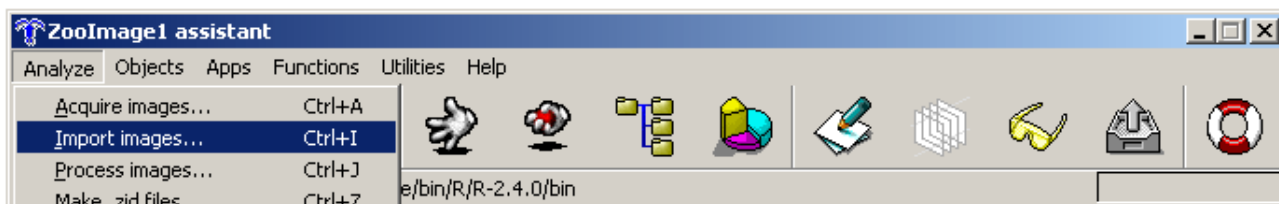
Now click on the second button on the toolbar, the one with the following icon:



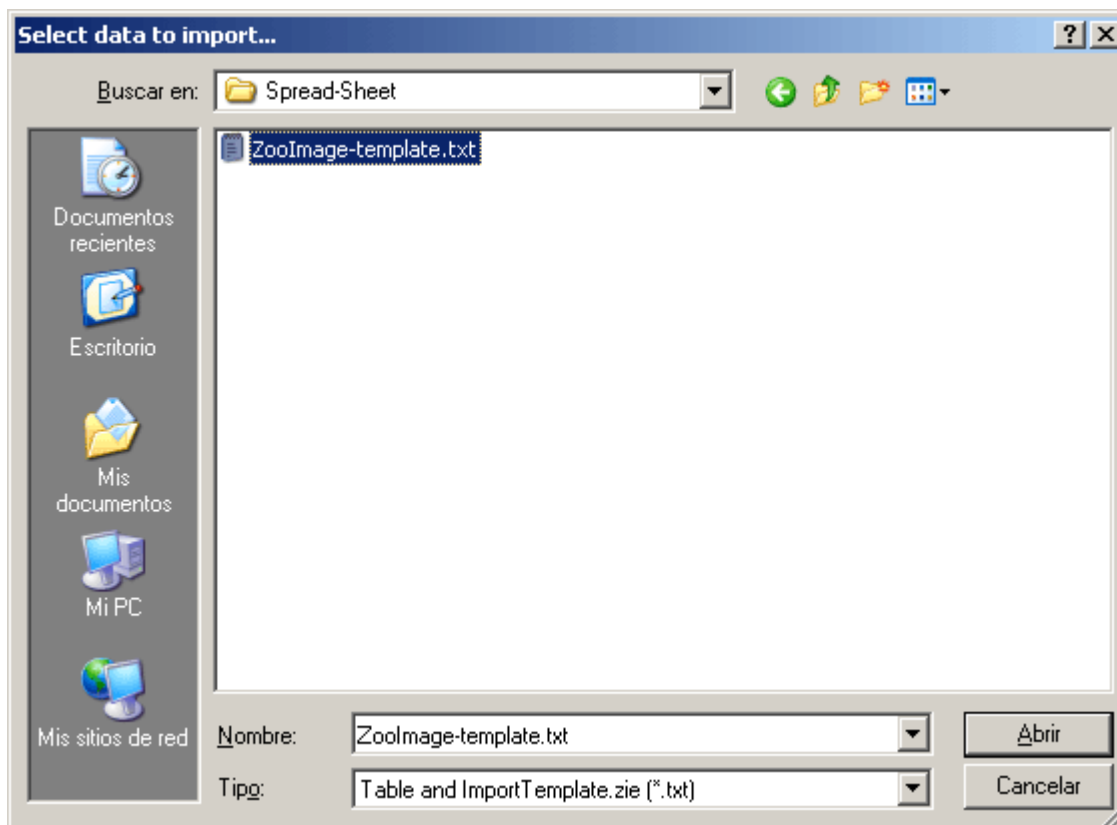
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Or select Analyze → Import images... in the menu.



ZooImage shows a ‘Select data to import...’ dialog box. First change type files of type field to ‘Table and importTemplate.zie (*.txt)’.



Then, select the table you just saved from Excel or OpenOffice Calc:

ZooImage processes the table, converts the images and automatically creates the associated metadata files (look at the activity printed in the ‘R Console’ window):



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```
R Console
File Edit Misc Packages Help ZooImage

Loading required package: utils
Loading required package: tcltk
Loading Tcl/Tk interface ... done
Loading required package: tcltk2
Loading required package: svMisc
Loading required package: svWidgets
Loading required package: svDialogs
Creating .zie file...
...OK!
Reading Filemap...
Checking all lines in the .zie file for raw images...
...OK!
Processing all lines in the .zie file (import images and make .zim files)...
...OK!
-- Done! --
> █
```

At the end of the process, a log file is displayed with a detail of the operations done, and possibly, explicit error messages in case of missing or wrong images for instance:



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```
R ZooImage1 log
File Edit

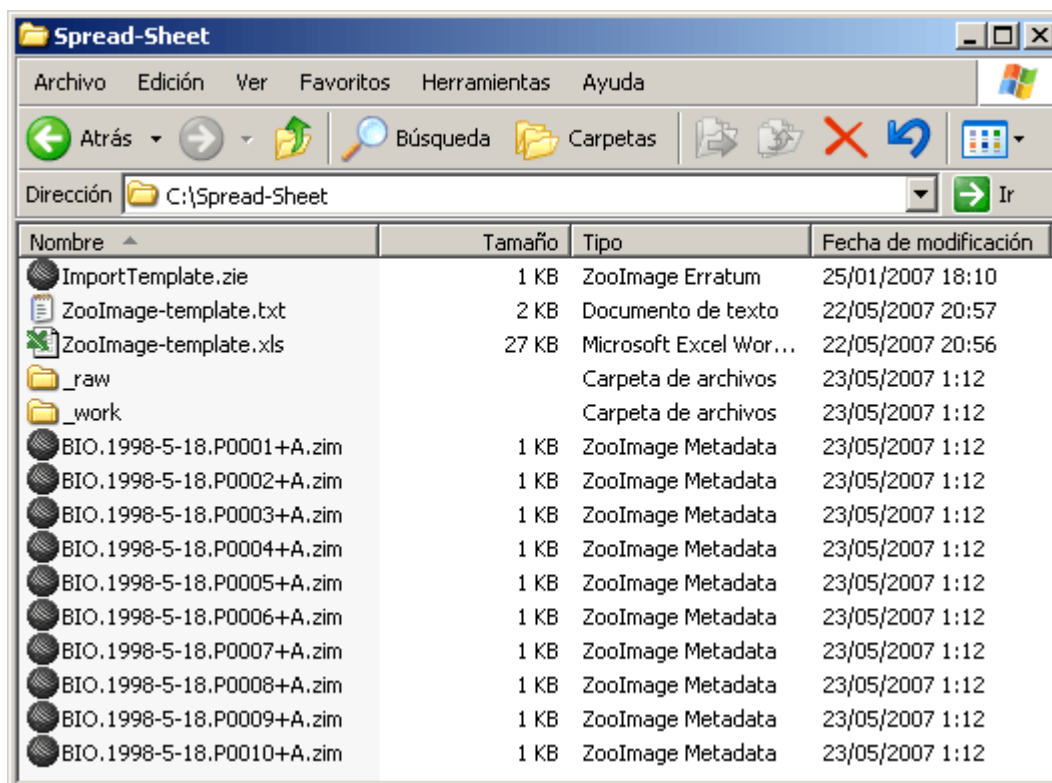
=== ZooImage1 log started 2007-05-23 01:11:49 ===

Creating .zie file...
Creating .zie file...
Reading Filemap... OK!
Checking all lines in the .zie file for raw images... OK!
Processing all lines in the .zie file (import images and make .zim files)...
Writing .zim file for sample 'BIO.1998-5-18.P0001+A'
Processing image 'p-0001.jpg'
Writing .zim file for sample 'BIO.1998-5-18.P0002+A'
Processing image 'p-0002.jpg'
Writing .zim file for sample 'BIO.1998-5-18.P0003+A'
Processing image 'p-0003.jpg'
Writing .zim file for sample 'BIO.1998-5-18.P0004+A'
Processing image 'p-0004.jpg'
Writing .zim file for sample 'BIO.1998-5-18.P0005+A'
Processing image 'p-0005.jpg'
Writing .zim file for sample 'BIO.1998-5-18.P0006+A'
Processing image 'p-0006.jpg'
Writing .zim file for sample 'BIO.1998-5-18.P0007+A'
Processing image 'p-0007.jpg'
Writing .zim file for sample 'BIO.1998-5-18.P0008+A'
Processing image 'p-0008.jpg'
Writing .zim file for sample 'BIO.1998-5-18.P0009+A'
Processing image 'p-0009.jpg'
Writing .zim file for sample 'BIO.1998-5-18.P0010+A'
Processing image 'p-0010.jpg'

-- OK, no error found. --
```

This is the typical way of working for ZooImage: the program is made to process in batch many images. You can leave the program unattended because it will give an informative message about problems encountered. Also, for most complex tasks, a first quick pass is programmed to check if all files and data are there, so that you can let it work during the night once this first pass is successful. Then, most of the time, the program can recover from an error and will simply process the following files without interruption.

At this stage, you have imported all your images into ZooImage. Here is what you have :



You can see that your images have disappeared from your directory. Instead place, you have P1Ex-01+A.zim and P1Ex-02+A.zim files, together with the new re-named images as .tif or .jpg, depending on the original source.

For the next step, it is necessary to have .jpg formatted images on this directory, specially if we want to process images in colour. In this case, we have to rename the images changing the extension from “.tif” to “.jpg”.

At the end, we should have (depending on the version of *Zooimage* this could appear slightly changed):

- .txt file
- .xls file
- .zim files
- .jpg files (or .tif)
- “_raw” and “_work” subdirectories:
 - Original images (.tif) (renamed, depending on version)



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- .zie: Original template and template plus data

Depending on the process and *Zooimage* version, the “_raw” or the “_work” directory is used during processing.

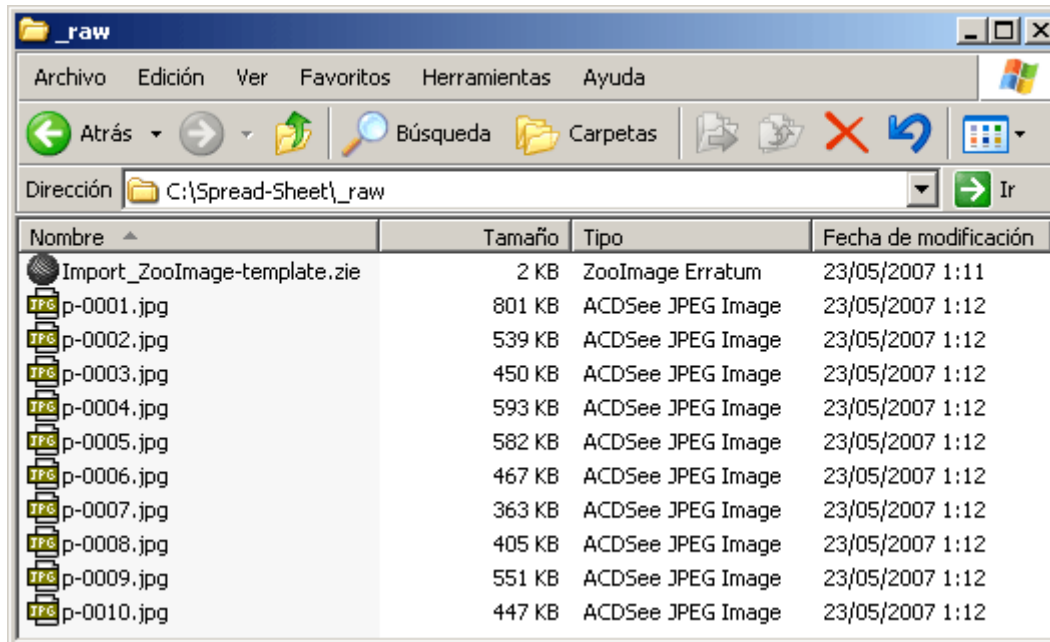
```
BIO.1998-5-18.P0001+A.zim
File Edit Search Project View Format Column Macro Advanced Window He
Zi1
[Image]
Author= AZTI - Tecnalia - Unidad de Investigación Marina
ImageType=trans 8bits color 600dpi
Hardware=hp Scanjet 8200
Software=Digital Imaging

[Import]
FilenamePattern=p-<4>.jpg
FractionPattern=
SubsamplePattern=
Convert=
Return=
FileExt=
FileConv=
FileExt2=.jpg
Nmin=5
Nmax=5

[Fraction]
Code=A
Min=-1
Max=-1

[Subsample]
SubPart=0.027777778
SubMethod=volumetry
CellPart=1.00
Replicates=1
VolIni=8.1207
VolPrec=
CellPart=1
```

These are the ZooImage Metadata files that give all the information required to further process the images. You have also two subdirectories: _raw and _work.



An Import_ZooImage-template.zie file was also compiled and created. It can be used instead of the Table + ImportTemplate.zie files to tell to ZooImage how to reimport those images.

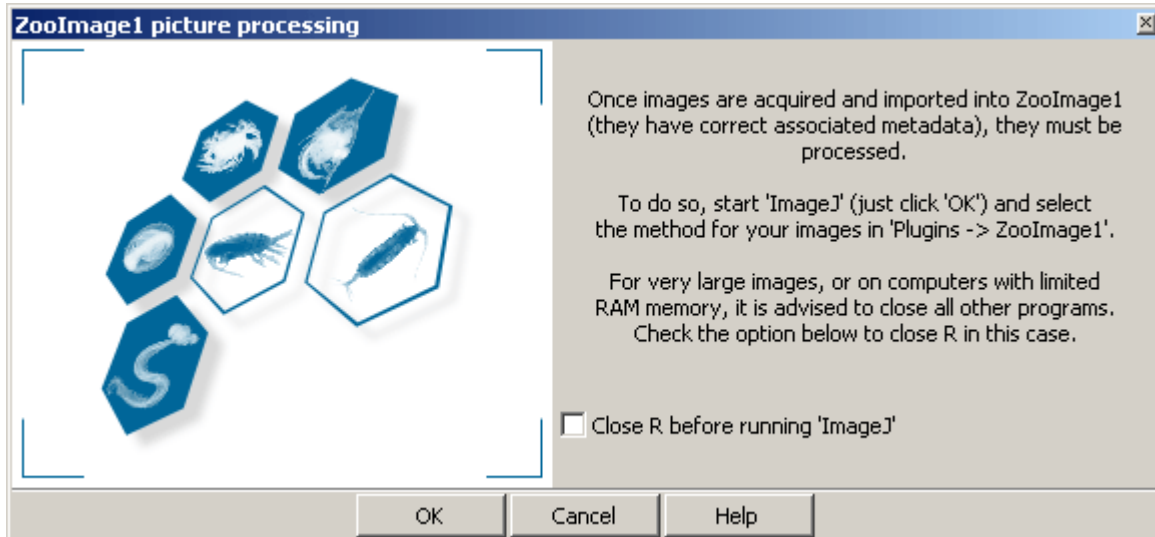
12.2.3. Process images



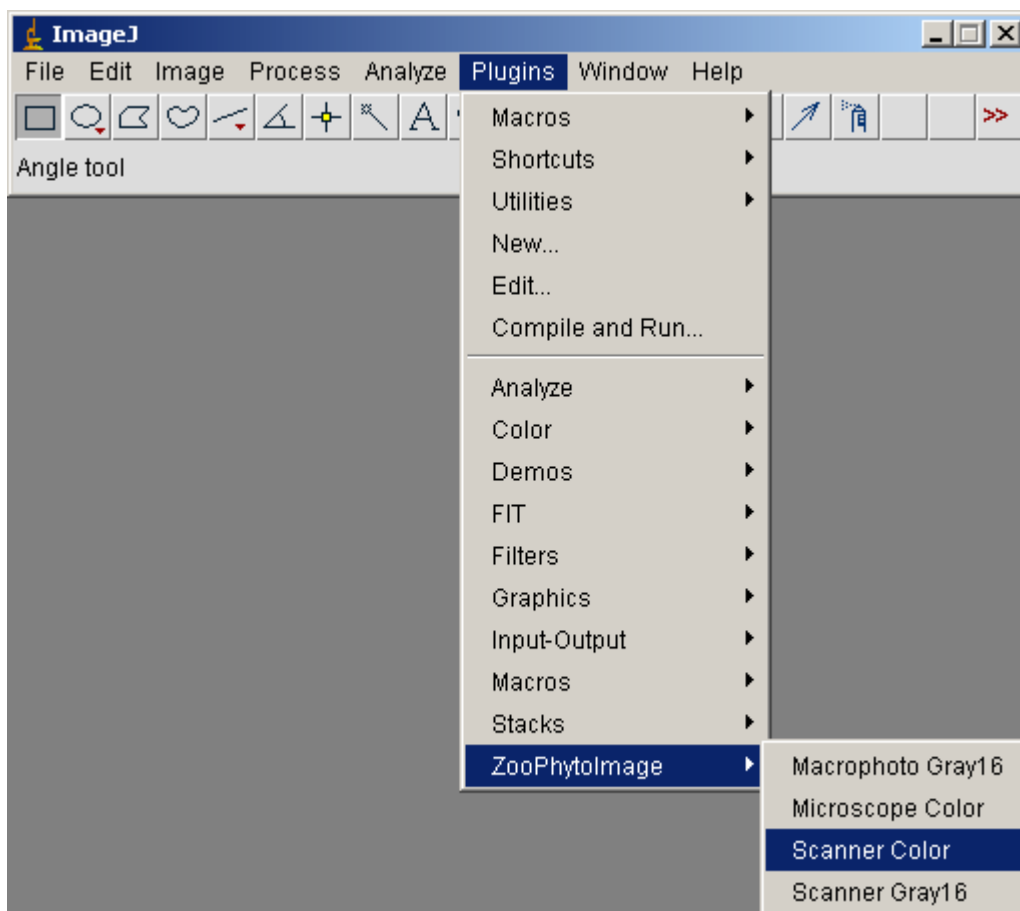
To process your images, use the menu entry Analyze → Process images..., the shortcut Ctrl+J, or click on the third button in the toolbar.



Then, a dialogue is displayed to advertise you to close R in case of computer with low Ram memory.

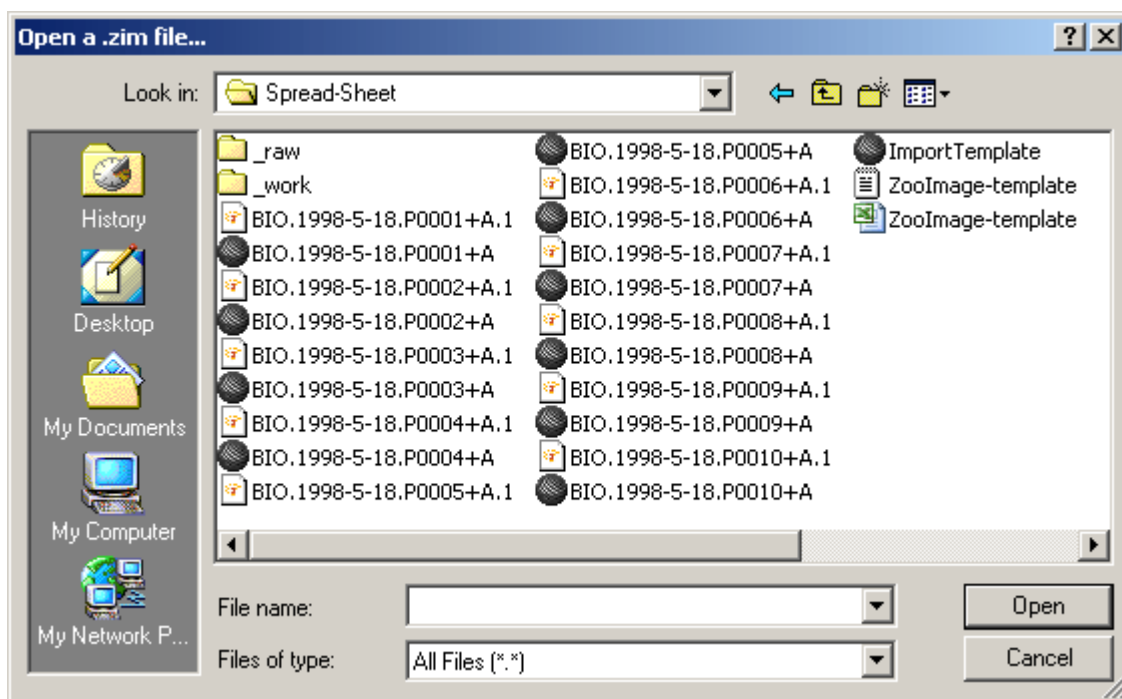


For our images, we have to select the Scanner Color plugin.



The plugin first asks you to select a .zim file. **Do not select on image file here.** The **zim**

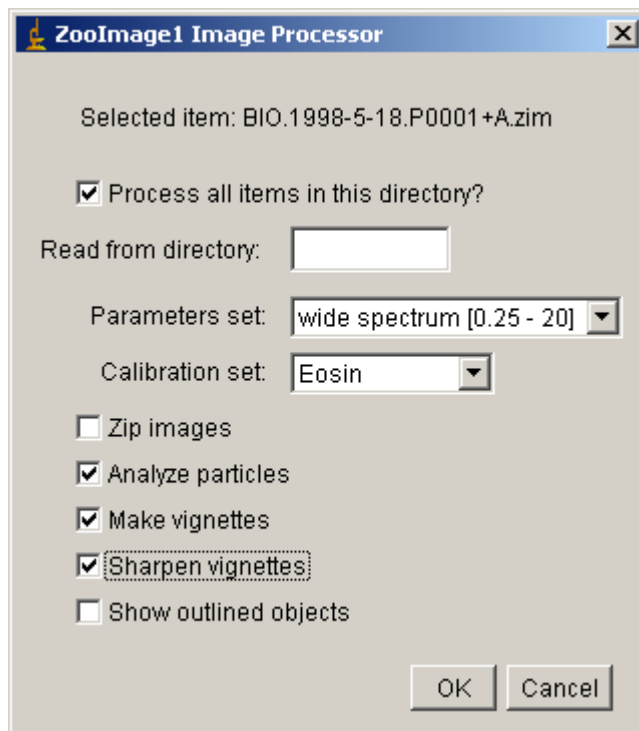
files and **renamed jpg images must be in the same directory**. Depending the version of ZooImage, the files might not be moved automatically and have to be done manually. It is recommended to move .jpg files to the directory where are the zim files, in this case the root 'C:\Spread-Sheet'.



The reasons why you have to select the .zim file instead of the corresponding image are:

- We are sure you have metadata associated with the image(s),
- As explained here above, you could have several images for the same sample/fraction. The plugin will process **all** images associated with the selected .zim file, not only one. In the example, we have only one image for each .zim file.

You then have a dialogue box with parameterization of your process:



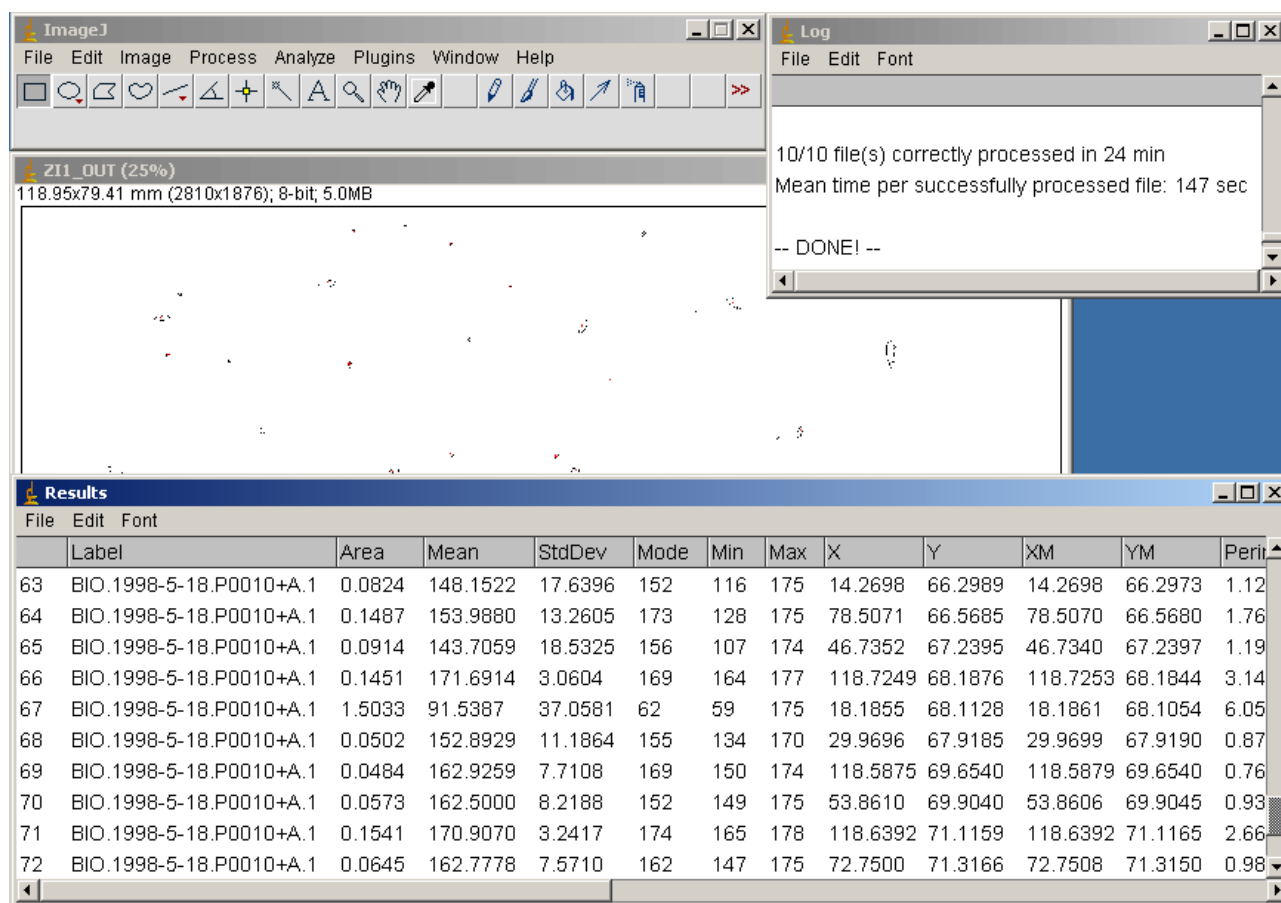
- You can **process all items in this directory** (all images that have associated .zim files), or only that one [*keep this checked now*].
- You can optionally **read images from a different directory**. This function is useful if you saved your large images on DVDs or external disks. You just have to copy the small associated .zim files in your process directory and you point to the directory that contains the images on your DVD [*leave this blank now*].
- **Analyze particles: Option that allows you to** do the measurements on the particles after processing the images [*leave this option checked now*].
- **Make vignettes: Option that allows you to** extract small images for each identified object, called ‘vignettes’ in PhytoImage’s terminology [*leave this option checked now*].
- **Sharpen vignettes: Option that allows you to** apply a “sharpen” filter on the pictures in the vignettes. This often enhances the quality of the vignettes, but is not necessary for some kinds of pictures [*leave this option checked now*].
- Show **outlined objects: Option that allows you to** display a composite image with the detected object outlines superposed to the grayscale image. This is a very useful diagnostic to determine if segmentation and detection of the objects was correct [*So, leave this option*

checked now].

*The **show outlined objects** option works only for the last picture processed. So, either uncheck **process all items in this directory**, or be prepared to wait for the last picture to get this diagnostic image! You should zoom in the image (Image → Zoom → 100% entry menu) and pan it by selecting the hand button and dragging the image content in the window to best see the result.*

When you start the process by clicking OK on the dialog box, ImageJ do the following work:

- It opens a Log window and reports its activity in it.
- It opens each image in turn, process it, and possibly measure particles and extract vignettes. You can follow the process on the screen. Note that a scale bar is added in the top-right corner of each vignette for convenience.
- It possibly displays the outlined objects of last picture if it was requested. Also, the last table of measurements is left open for inspection.



The screenshot shows the ImageJ software interface. The main window displays an image titled 'Z11_OUT (25%)' with dimensions 118.95x79.41 mm (2810x1876), 8-bit, 5.0MB. The Log window shows the following text:

```
10/10 file(s) correctly processed in 24 min
Mean time per successfully processed file: 147 sec
-- DONE! --
```

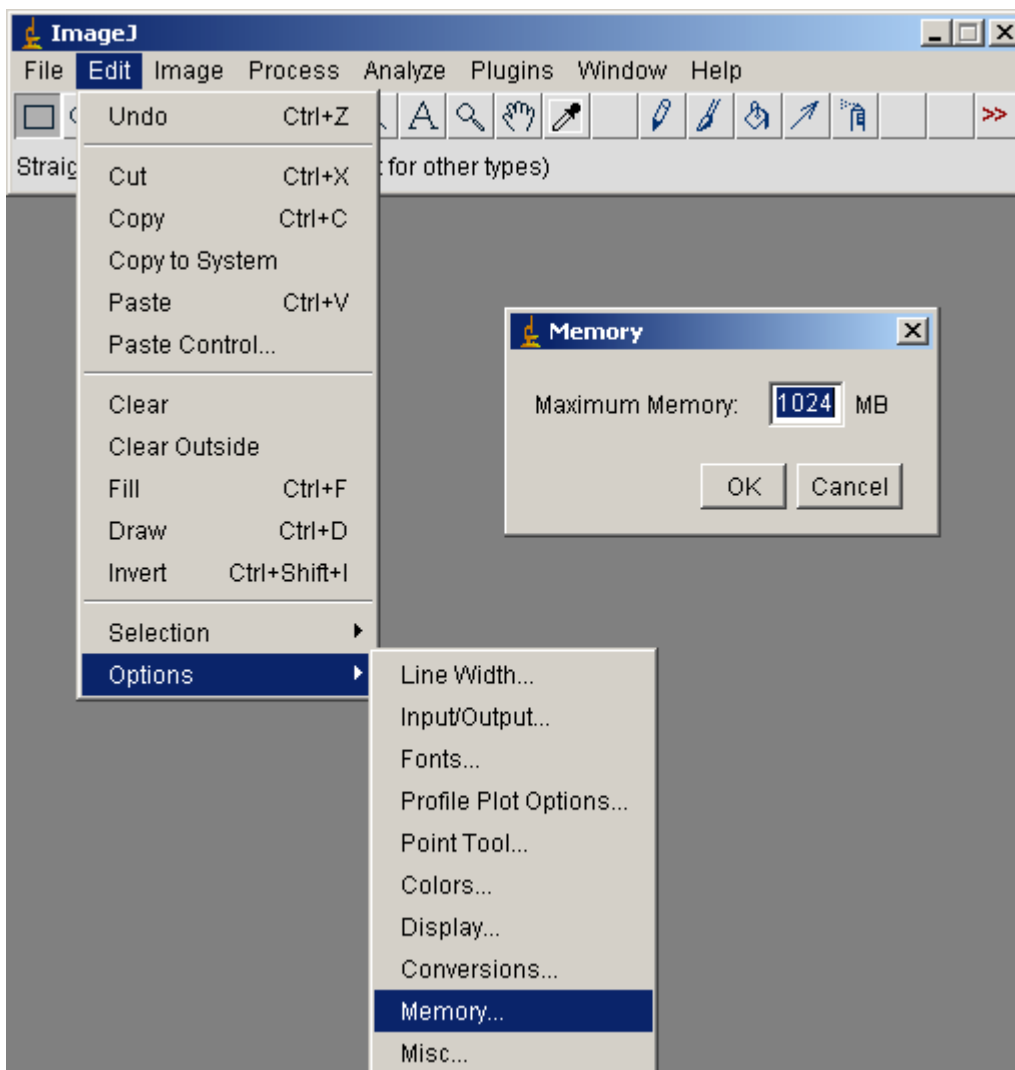
The Results window displays a table of measurements for 10 files:

File	Label	Area	Mean	StdDev	Mode	Min	Max	X	Y	XM	YM	Perim
63	BIO.1998-5-18.P0010+A.1	0.0824	148.1522	17.6396	152	116	175	14.2698	66.2989	14.2698	66.2973	1.12
64	BIO.1998-5-18.P0010+A.1	0.1487	153.9880	13.2605	173	128	175	78.5071	66.5685	78.5070	66.5680	1.76
65	BIO.1998-5-18.P0010+A.1	0.0914	143.7059	18.5325	156	107	174	46.7352	67.2395	46.7340	67.2397	1.19
66	BIO.1998-5-18.P0010+A.1	0.1451	171.6914	3.0604	169	164	177	118.7249	68.1876	118.7253	68.1844	3.14
67	BIO.1998-5-18.P0010+A.1	1.5033	91.5387	37.0581	62	59	175	18.1855	68.1128	18.1861	68.1054	6.05
68	BIO.1998-5-18.P0010+A.1	0.0502	152.8929	11.1864	155	134	170	29.9696	67.9185	29.9699	67.9190	0.87
69	BIO.1998-5-18.P0010+A.1	0.0484	162.9259	7.7108	169	150	174	118.5875	69.6540	118.5879	69.6540	0.76
70	BIO.1998-5-18.P0010+A.1	0.0573	162.5000	8.2188	152	149	175	53.8610	69.9040	53.8606	69.9045	0.93
71	BIO.1998-5-18.P0010+A.1	0.1541	170.9070	3.2417	174	165	178	118.6392	71.1159	118.6392	71.1165	2.66
72	BIO.1998-5-18.P0010+A.1	0.0645	162.7778	7.5710	162	147	175	72.7500	71.3166	72.7508	71.3150	0.98

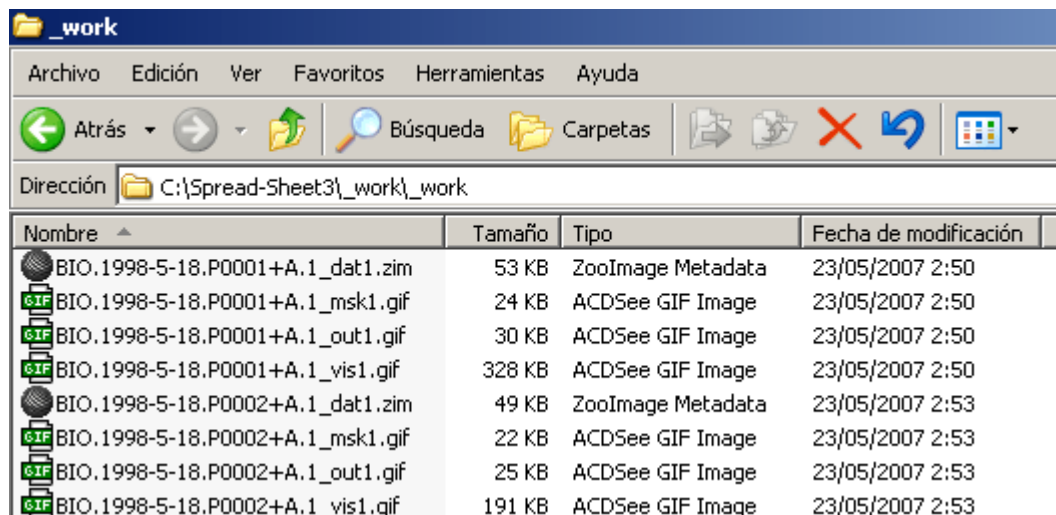
IF ANY PROBLEM APPEARS DURING THIS PROCESS:

***If the process failed somewhere** look if your images are of the right type, if they are not too big for the RAM memory allocated and if the correct plug-in, parameters set and calibration set where selected. Look at the log file and the images produced in the _work directory to help you track the problem.*

***If you receive a message with “out of memory”** then you must download the last version of ImageJ, actually the one that corrects this “leaking problem” is the beta version, not the last official one. In addition, the memory available to ImageJ can be increased. It is not recommendable to reserve more than 2/3 of Ram available. Select in the menu Edit → Options → Memory...:*



The plug-ins created several files in your `_work` subdirectory for each processed image:



In this work directory, are created several images and file:

BIO.1998-5-18.P0001+A.1_dat1.zim: Initial raw image renamed during importation.

BIO.1998-5-18.P0001+A.1_msk1.gif: Binary image with the mask used to detect the silhouette of the particles.

BIO.1998-5-18.P0001+A.1_out1.gif: Image with the outline and labels of all particles that are retained after applying a minsize/maxsize filter.

BIO.1998-5-18.P0001+A.1_vis1.gif: 'Visual' image (used to make vignettes for visual identification of the particle by taxonomists).

BIO.1998-5-18.P0001+A.1_dat1.zim: `_dat1.zim` files associated for each image with all metadata and measurements associated with the image(s).



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BIO.1998-5-18.P0001+A.1_dat1.zim - Sc1

File Edit Search View Tools Options Language Buffers Help

FileExt2=.jpg
 Nmin=5
 Nmax=5

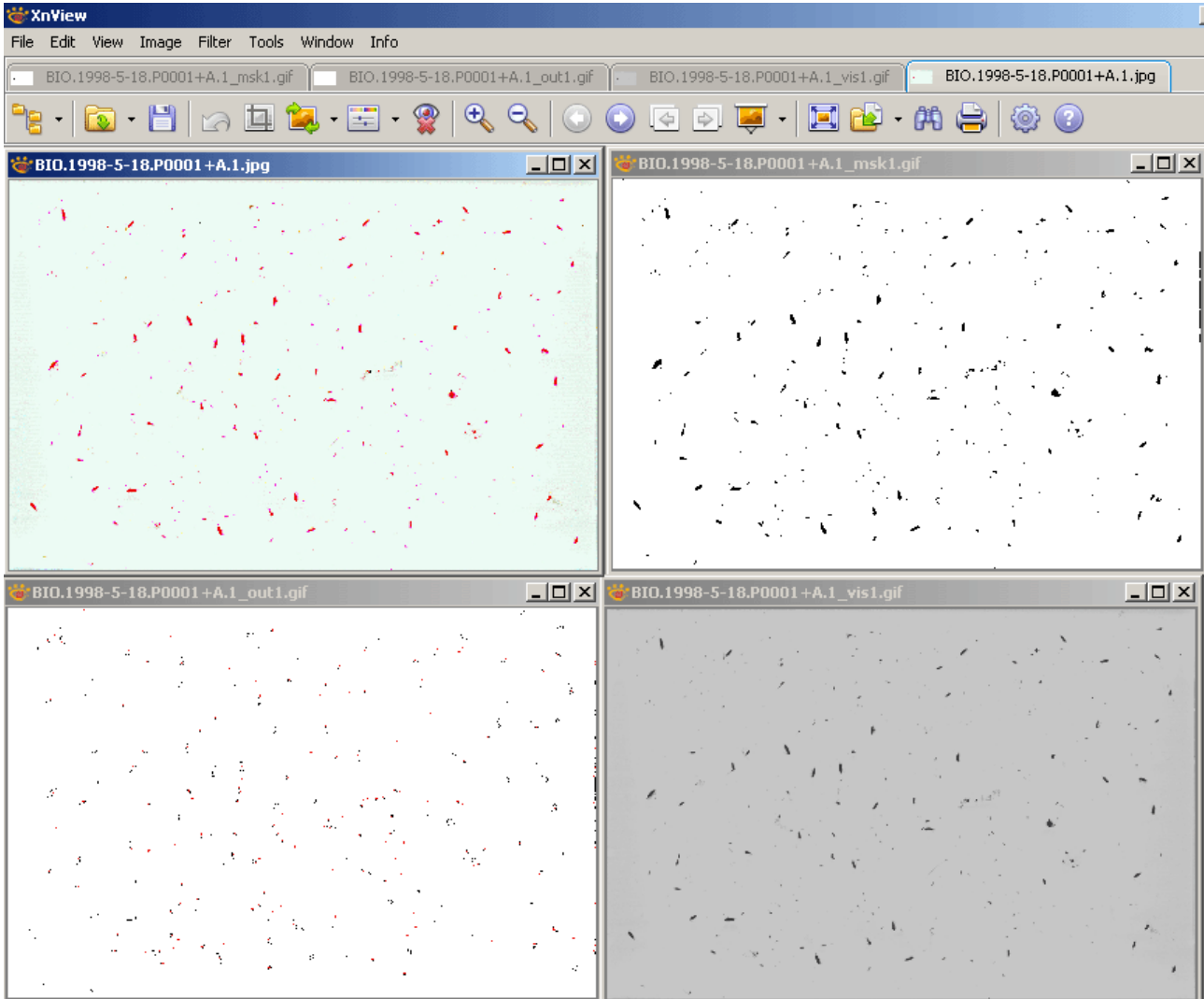
[Fraction]
 Code=A
 Min=-1
 Max=-1

[Subsample]
 SubPart=0.027777778
 SubMethod=volumetry
 CellPart=1.00
 Replicates=1
 VolIni=8.1207
 VolPrec=
 CellPart=1

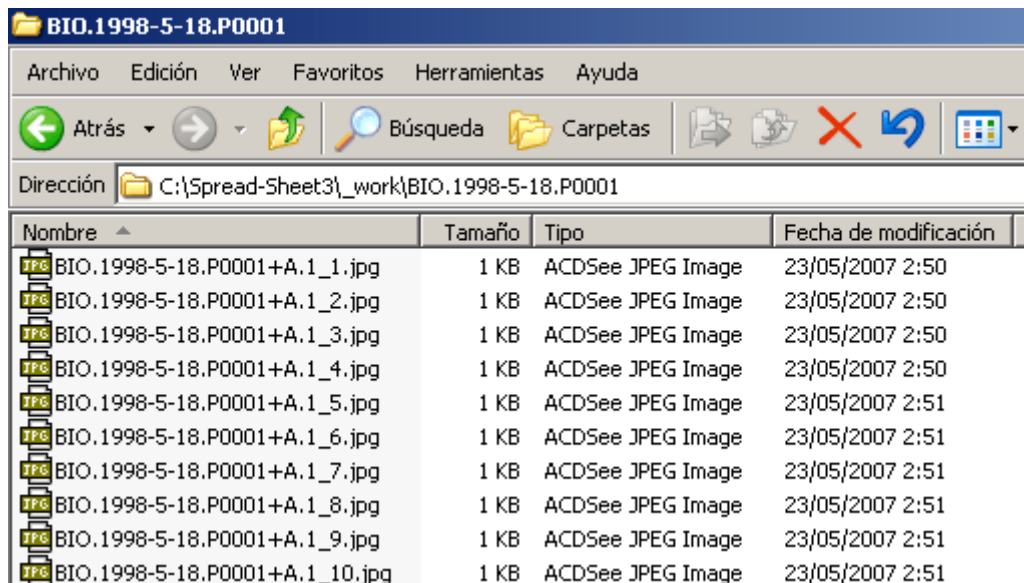
[Process]
 Version=1.0-0
 Method=wide spectrum [0.25 - 20]
 MinSize=0.25
 MaxSize=20.0
 Calibration=Eosin
 ProcessPixSize=0.04233
 ProcessPixUnit=mm
 Staining=haematoxylyn
 RedCoef=0.1
 BlueCoef=0.9
 GreenCoef=0.8
 Threshold=125

[Data]

!Item	Label	Area	Mean	StdC
1	→BIO.1998-5-18.P0001+A.1	0.1021	168.8772	4.91
2	→BIO.1998-5-18.P0001+A.1	0.0520	145.8276	22.2
3	→BIO.1998-5-18.P0001+A.1	0.0753	154.4286	14.0
4	→BIO.1998-5-18.P0001+A.1	0.5053	118.4007	28.2
5	→BIO.1998-5-18.P0001+A.1	0.0502	144.6429	20.2
6	→BIO.1998-5-18.P0001+A.1	0.1451	139.3704	22.5
7	→BIO.1998-5-18.P0001+A.1	0.0555	152.7419	18.1

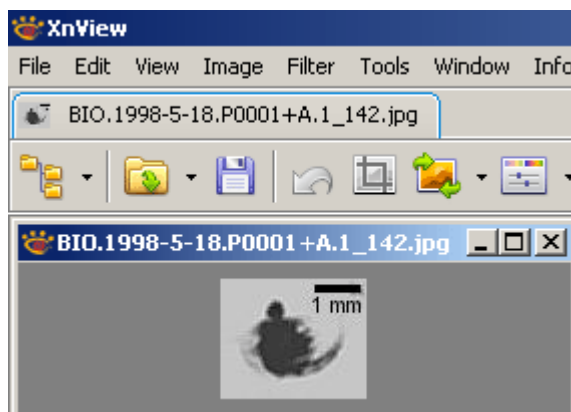


The four images in the _work subdirectory for each initial image after processing them. From left to right and top to bottom: the original image, the mask, the outline and the visual.



The program also creates an additional subdirectory for each sample. It places a copy of all the related _dat1.zim files (metadata + measurements on all particles) and a series of xxxx_yy.jpg files called 'vignettes', that are small-pictures of each particle with a scale added at the top right of it.

Once the image processing is done, you can close ImageJ and return to ZooImage.



12.2.4. Create ZID files



To finalize your images import/process, you must now build .zid files. In the PhytoImage1 assistant, use the menu entry Analyze → Make .zid files..., the shortcut Ctrl+Z, or click on the fourth button in the toolbar.

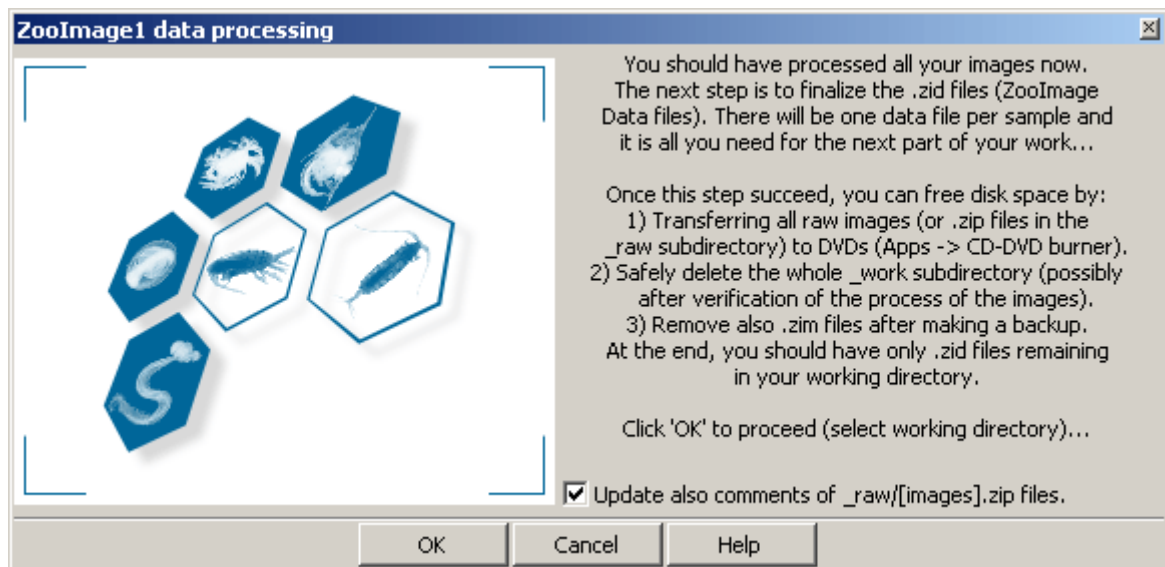


You have now to create the **.zid files**. These are special *ZooImage/PhytoImage Data* files that contain all you need for the rest of the analysis, but saves as much disk space as possible.

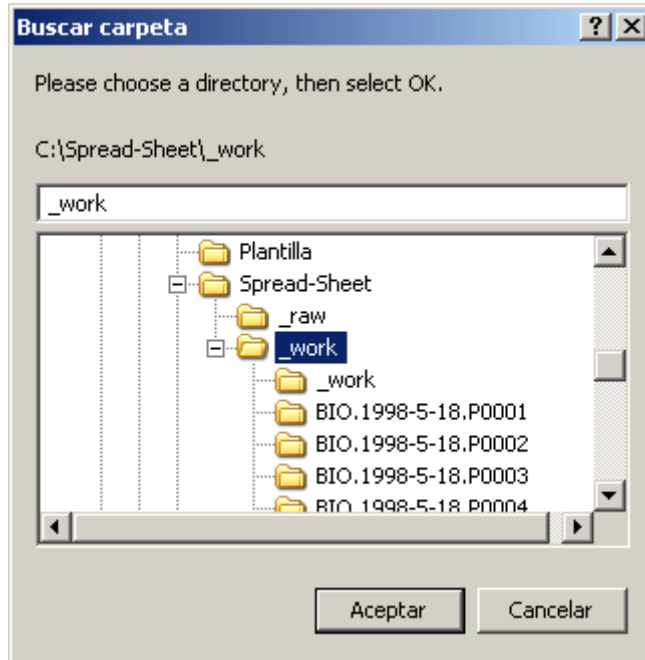
Now, click on the fourth button in the *PhytoImage1* assistant:



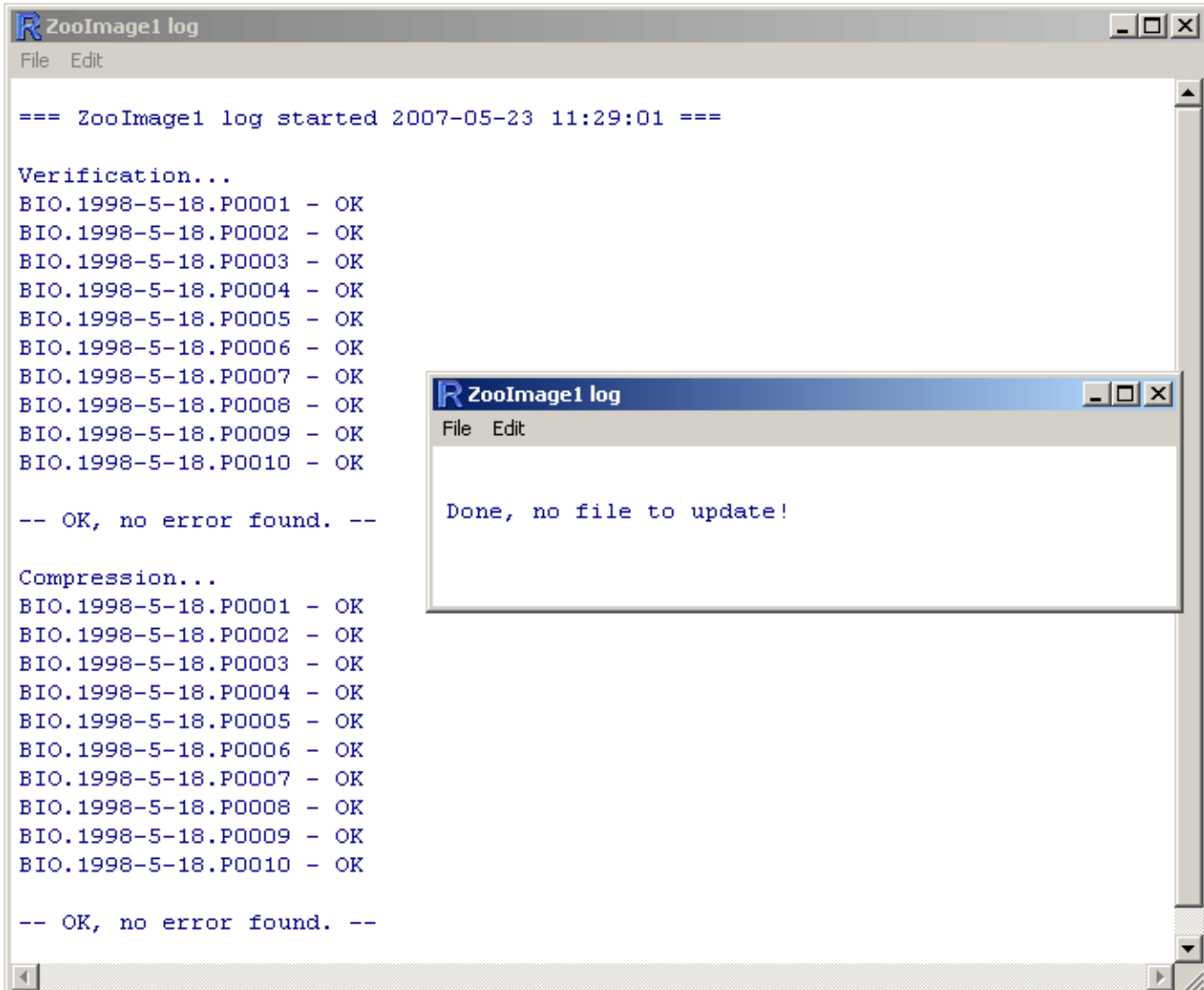
This shows the following dialogue box:



By clicking OK, you compute **.zid files** for your processed samples. The option **update also comments of _raw/[images].zip files** add **.zim** data as comments to zipped image files (if you selected that option in the process). [*Since we did not zipped images, we should uncheck that option now and click OK*]. You are prompted for a directory where treated data are located. Select **“Spread-Sheet”** (C:\Spread-Sheet):



ZooImage calculates .zid files and issues a report at the end of the process. For convenience, it first carries out the verification of files just created. Stay in front of the computer during checking. **Make sure there is no error reported once the compression is done.**

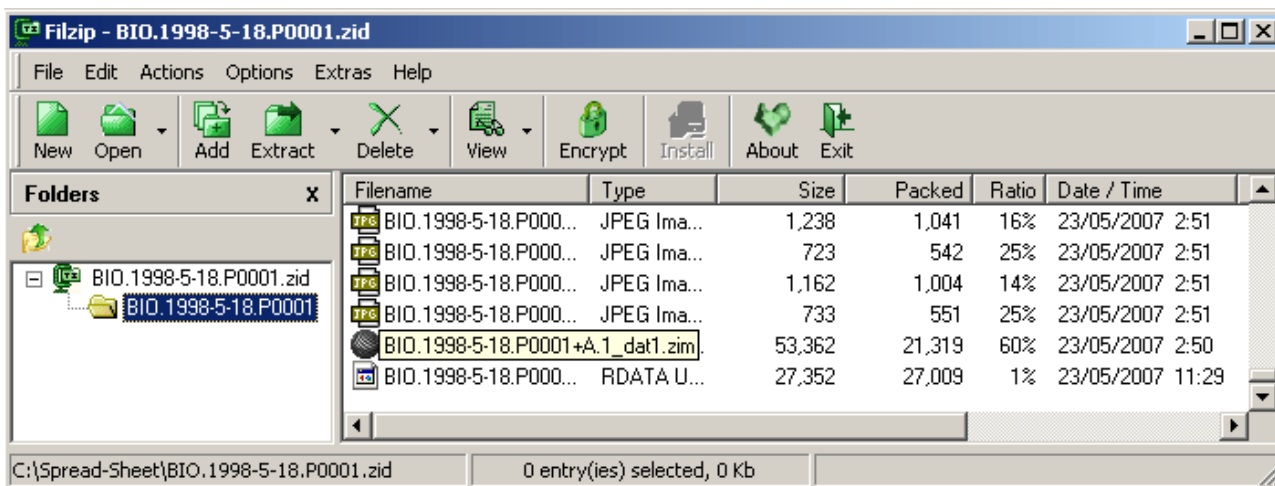


Cleaning the hard disk at the end of the process.

In other tutorials there is a cleaning process explained, but because this is a specific process based in the practices at Azti, here will be no cleaning process. Instead we recommend to copy everything to an external disk for future work. And have a second one has backup. This copies can be unattended using Cobian Free Software. Cobian permits to do sincronization of folders, schedule backups and deals well with long names.

FALTA TUTORIAL COBIAN.

Note that .zid files are a special kind of zipped archives that contain all PhytoImage needs to work with one sample: the _dat1.zim files, all the vignettes, and a dat1.Rdata (compilation of all the data in R format). You can, thus, inspect .zid files easily with programs like WinZip, for instance:



12.2.5 Manually classifying vignettes

In order to train the computer to (semi)-automatically recognize phytoplankton taxa on the basis of images measurements done in PhytoImage, you have to make a manual training set. Unfortunately, the 'PhytoImage-example' training does not have enough particles to make a valid training set. If you have access to the IFREMER report and the associated training set, you can follow instructions in SpainBioman tutorial to make a phytoplankton training set with these data. Otherwise, you can switch to the SpainBioman tutorial (**Chapter 12.1**), the principle remains exactly the same for phytoplankton in PhytoImage.

12.2.6. Make training set

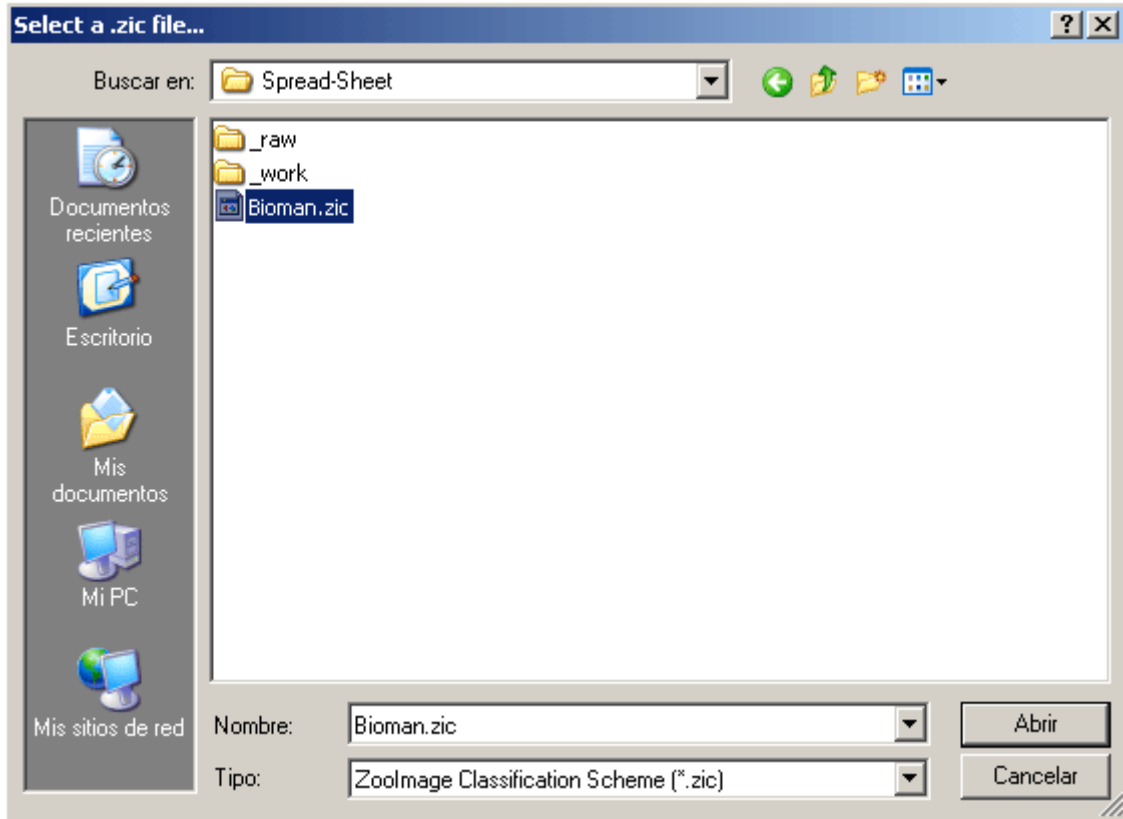


To make the training set, use the menu entry Analyze → Make training set..., the shortcut Ctrl+M, or click on the fifth button in the toolbar.

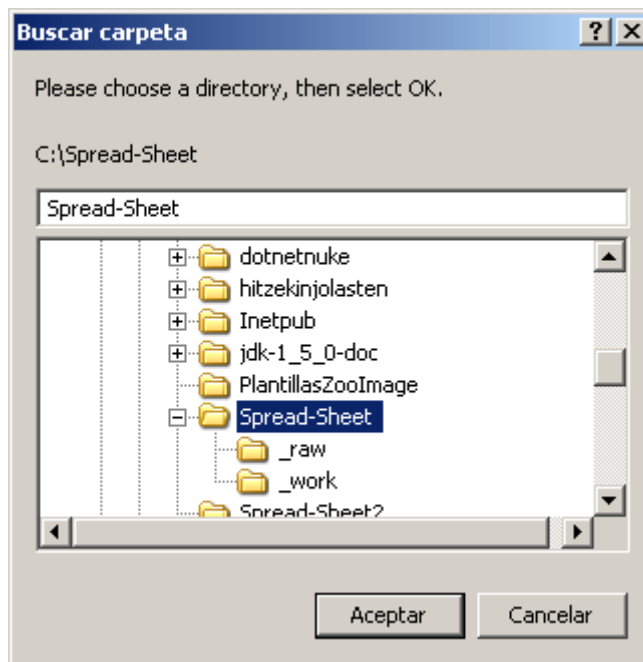


We are going to choose the option of Another config... to use a specification of classes adjusted to Bay of Biscay. It can be modified later if necessary.

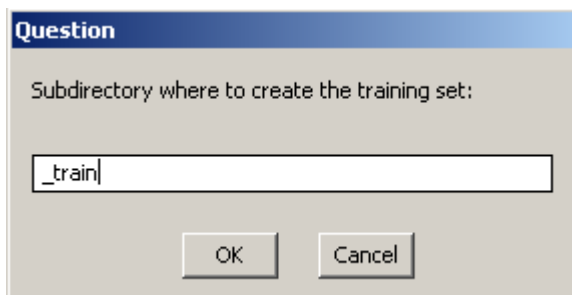
There is a predefined file between the templates that have to be selected:



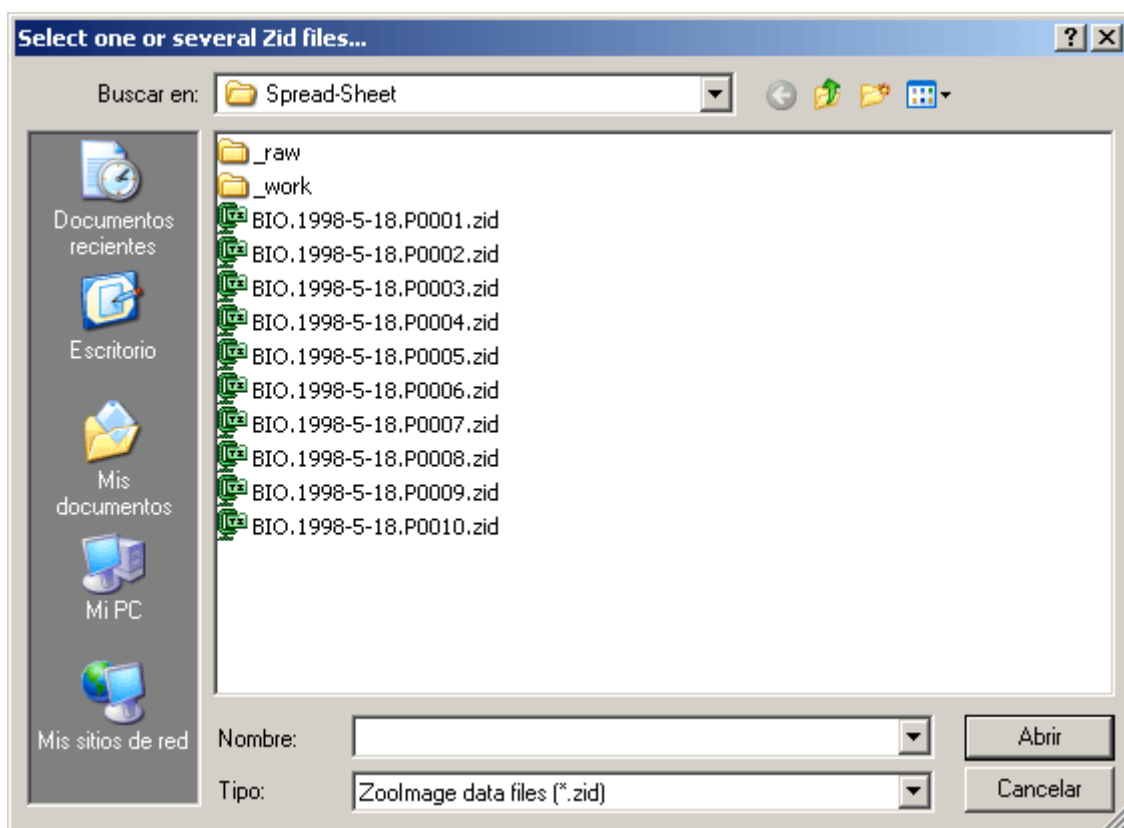
After that the folder where to place the training set have to be selected:



And give a name to the folder, by defect `_train`:



And finally select the Zid files with the vignettes that are going to be used for make the training set, in our case we will have preselected the most interesting ones doing an visual inspection of the original .jpg:



Because in this example there is only 10 images, all will be selected, after the process look at log files to see if all were processed successfully:



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```
ZooImage1 log
File Edit

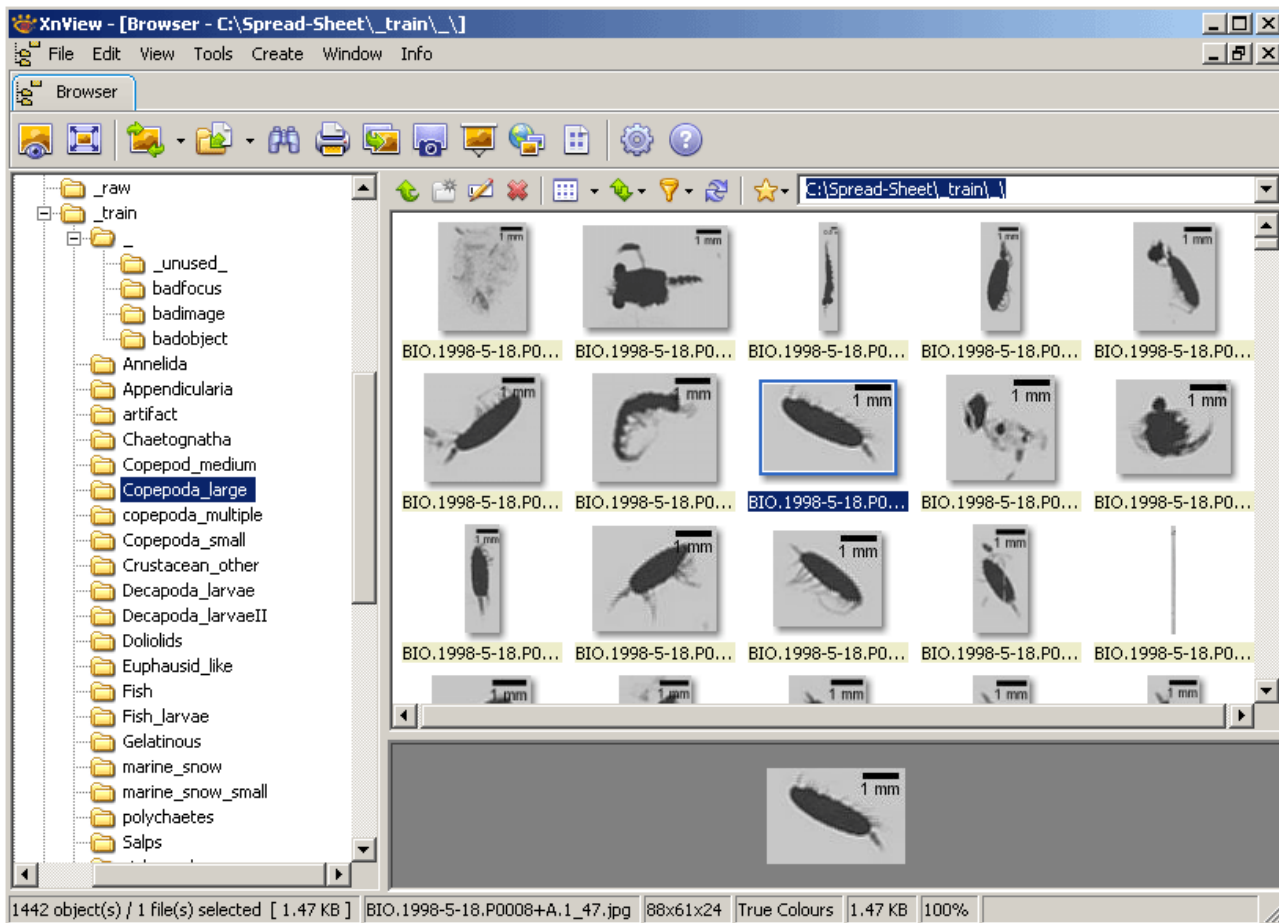
=== ZooImage1 log started 2007-05-23 17:54:33 ===

Extracting data...
C:\Spread-Sheet\BIO.1998-5-18.P0010.zid - data
C:\Spread-Sheet\BIO.1998-5-18.P0001.zid - data
C:\Spread-Sheet\BIO.1998-5-18.P0002.zid - data
C:\Spread-Sheet\BIO.1998-5-18.P0003.zid - data
C:\Spread-Sheet\BIO.1998-5-18.P0004.zid - data
C:\Spread-Sheet\BIO.1998-5-18.P0005.zid - data
C:\Spread-Sheet\BIO.1998-5-18.P0006.zid - data
C:\Spread-Sheet\BIO.1998-5-18.P0007.zid - data
C:\Spread-Sheet\BIO.1998-5-18.P0008.zid - data
C:\Spread-Sheet\BIO.1998-5-18.P0009.zid - data

Extracting vignettes...
C:\Spread-Sheet\BIO.1998-5-18.P0010.zid - vignettes
C:\Spread-Sheet\BIO.1998-5-18.P0001.zid - vignettes
C:\Spread-Sheet\BIO.1998-5-18.P0002.zid - vignettes
C:\Spread-Sheet\BIO.1998-5-18.P0003.zid - vignettes
C:\Spread-Sheet\BIO.1998-5-18.P0004.zid - vignettes
C:\Spread-Sheet\BIO.1998-5-18.P0005.zid - vignettes
C:\Spread-Sheet\BIO.1998-5-18.P0006.zid - vignettes
C:\Spread-Sheet\BIO.1998-5-18.P0007.zid - vignettes
C:\Spread-Sheet\BIO.1998-5-18.P0008.zid - vignettes
C:\Spread-Sheet\BIO.1998-5-18.P0009.zid - vignettes

Making directories...
C:/Spread-Sheet/_train/_/_unused_
C:/Spread-Sheet/_train/_/badfocus
C:/Spread-Sheet/_train/_/badimage
```

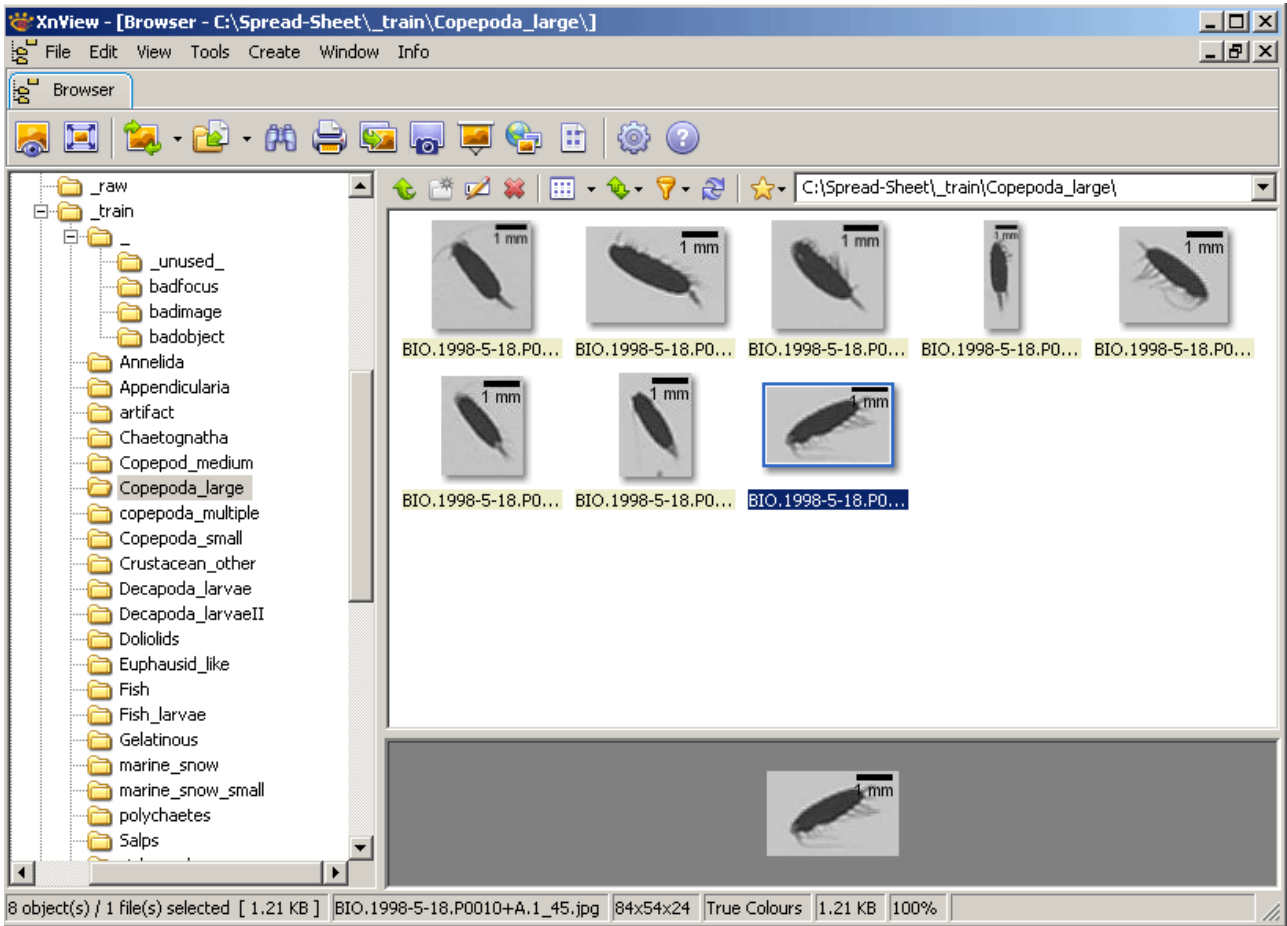
The XnView will open to be able to arrange the different particles in their class. Just move the particle or vignette to the corresponding folder, do click on the image and without leaving the button drag the image to the appropriate folder:



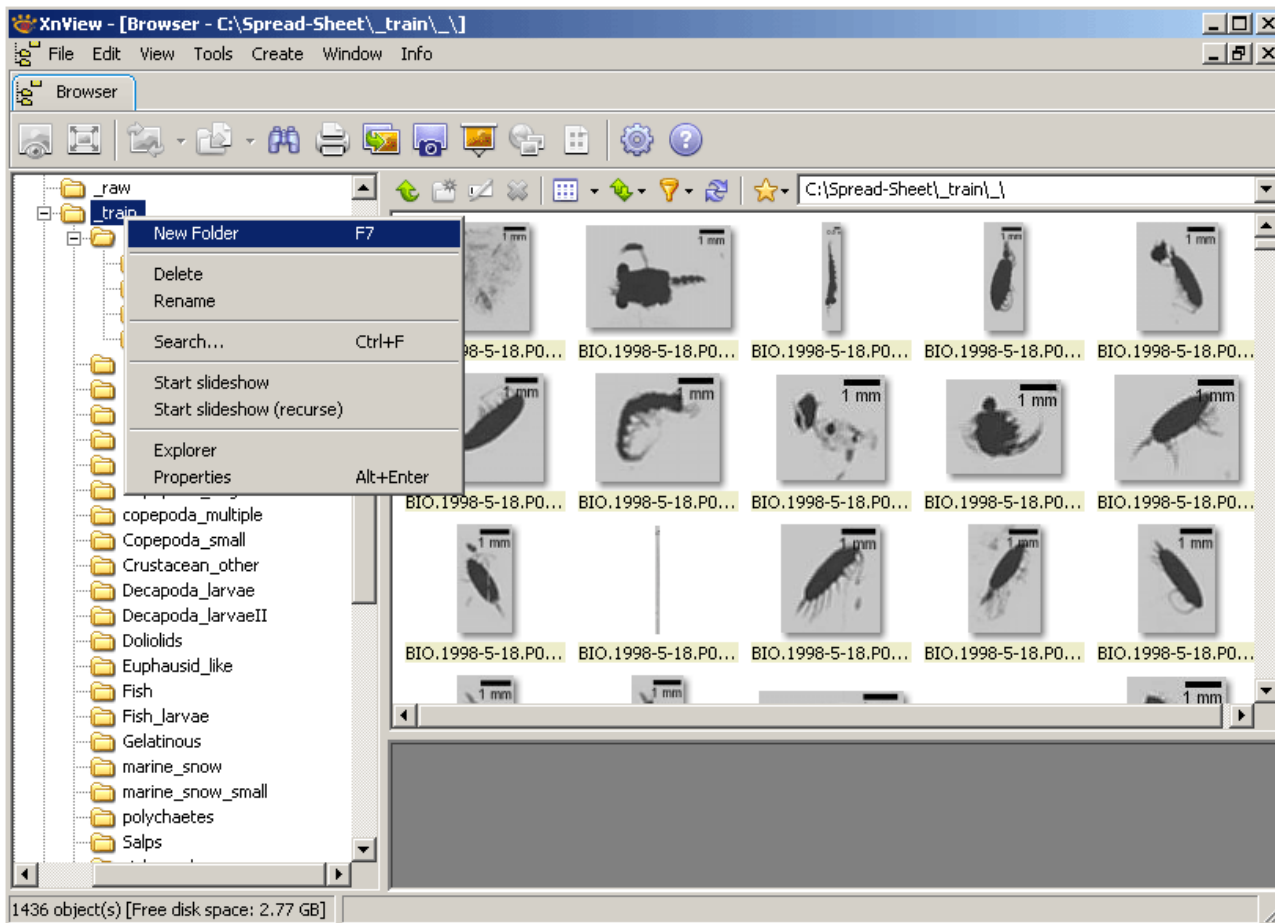
To revise the particles classified, click in the folder of the class:



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If you want to make a new class, you only have to make a folder for it:



Once you have finish, just close XnView. You can reopen it if you did not finish executing:
 C:\Archivos de programa\ZooImage\bin\XnView\ xnview.exe

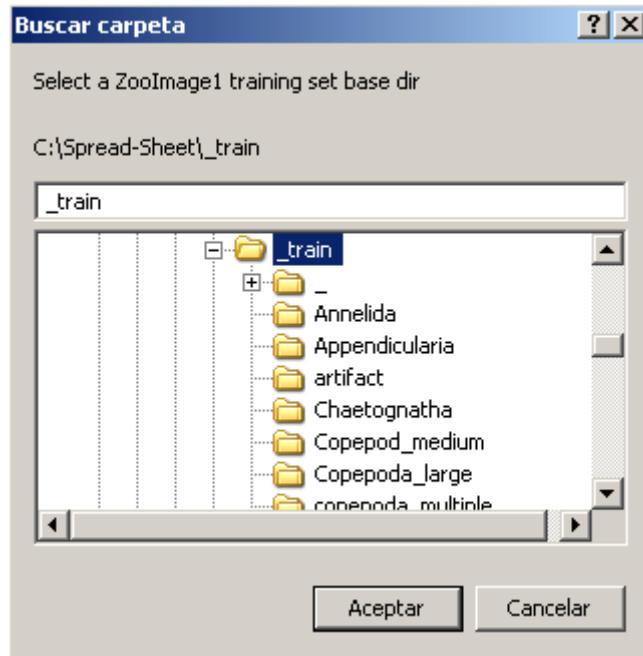
12.2.7 Reading training set



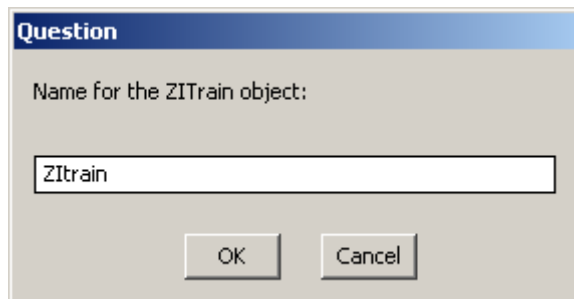
In order to do a classifier the training set data must be read, use the menu entry Analyze → Read training set..., the shortcut Ctrl+T, or click on the sixth button in the toolbar.



Select the folder where training set was previously created:



Give a name to the object that will be created in R:



After loading, statistics of the ZooPlankton classes are shown:

```

R Console
File Edit Misc Packages Help ZooImage

Classification stats:

      artifact      Copepod_medium      Copepoda_large      copepoda_multiple
           72              152              129              8
Copepoda_small      Decapoda_larvae      Euphausid_like      marine_snow
           46              3              9              13
Zooplankton_round  Zooplankton_small
           15              85

Proportions per class:

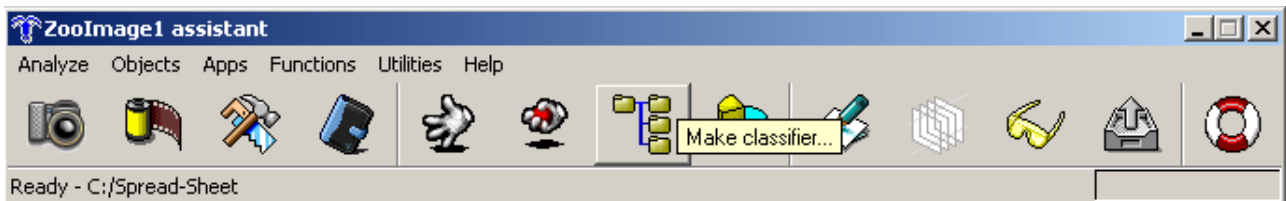
      artifact      Copepod_medium      Copepoda_large      copepoda_multiple
    13.5338346      28.5714286      24.2481203      1.5037594
Copepoda_small      Decapoda_larvae      Euphausid_like      marine_snow
     8.6466165      0.5639098      1.6917293      2.4436090
Zooplankton_round  Zooplankton_small
     2.8195489      15.9774436

>
  
```

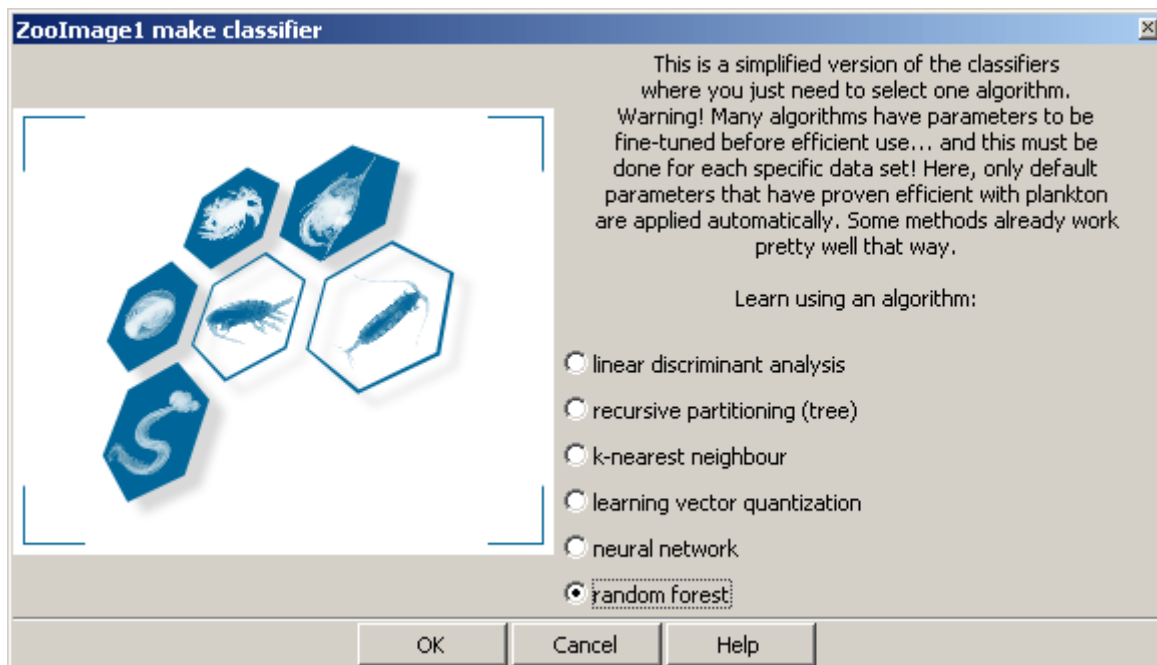
12.2.8 Make classifier



Now a classifier can be created, use the menu entry Analyze → Make classifier..., the shortcut Ctrl+C, or click on the seventh button in the toolbar.



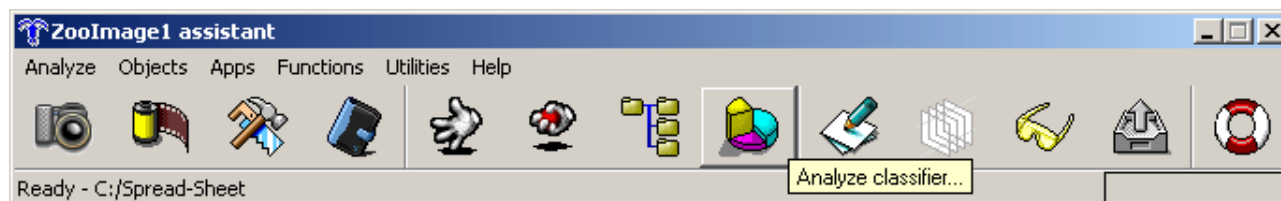
Then the type of classifier must be specified. For different problems one can be better than other. I recommend to choose Random Forest or k-nearest neighbour:



12.2.9 Analyse Classifier



ZooImage have this utility to see a confusion matrix to evaluate how 'good' is the classifier. An additional tool is being developed. use the menu entry Analyze → Make classifier..., the shortcut Ctrl+C, or click on the seventh button in the toolbar.



The confusion matrix helps to evaluate the classifier. If the error is big or there is a lot of confusion, the training set must be done. The diagonal are the instances well classified:



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```

R Console
File Edit Misc Packages Help ZooImage

Manual training set data collected in 'ZITrain'

Classification stats:

      artifact      Copepod_medium      Copepoda_large      copepoda_multiple
      72             152                 129                 8
      Copepoda_small  Decapoda_larvae      Euphausid_like      marine_snow
      46             3                 9                 13
      Zooplankton_round  Zooplankton_small
      15             85

Proportions per class:

      artifact      Copepod_medium      Copepoda_large      copepoda_multiple
      13.5338346     28.5714286         24.2481203         1.5037594
      Copepoda_small  Decapoda_larvae      Euphausid_like      marine_snow
      8.6466165     0.5639098         1.6917293         2.4436090
      Zooplankton_round  Zooplankton_small
      2.8195489     15.9774436

Erro en analyzeClass() : No current classifier. Please, make one first!
>
  
```

12.2.10 Edit Samples description



Instead of use this facility, there is an alternative similar to zim files. Open the xls file, and fill the second sheet called ForZis. Some field are generated automatically to save time and to avoid error. Be careful with dates, must be in format yyyy-mm-dd or ZooImage will crash and you will not know why:

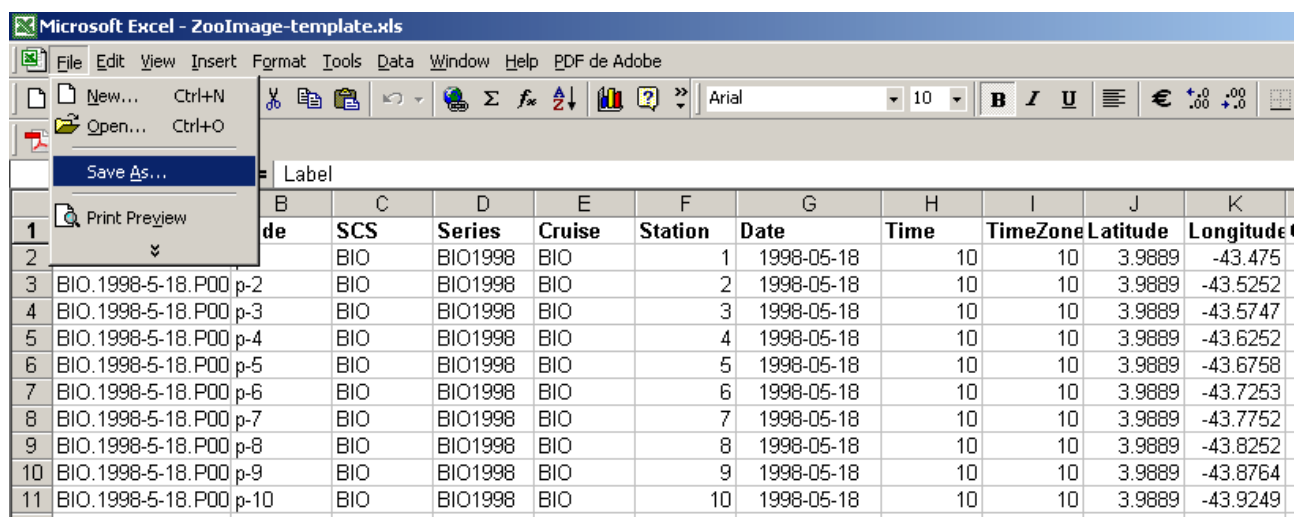
Microsoft Excel - ZooImage-template.xls

File Edit View Insert Format Tools Data Window Help PDF de Adobe

A1 = Label

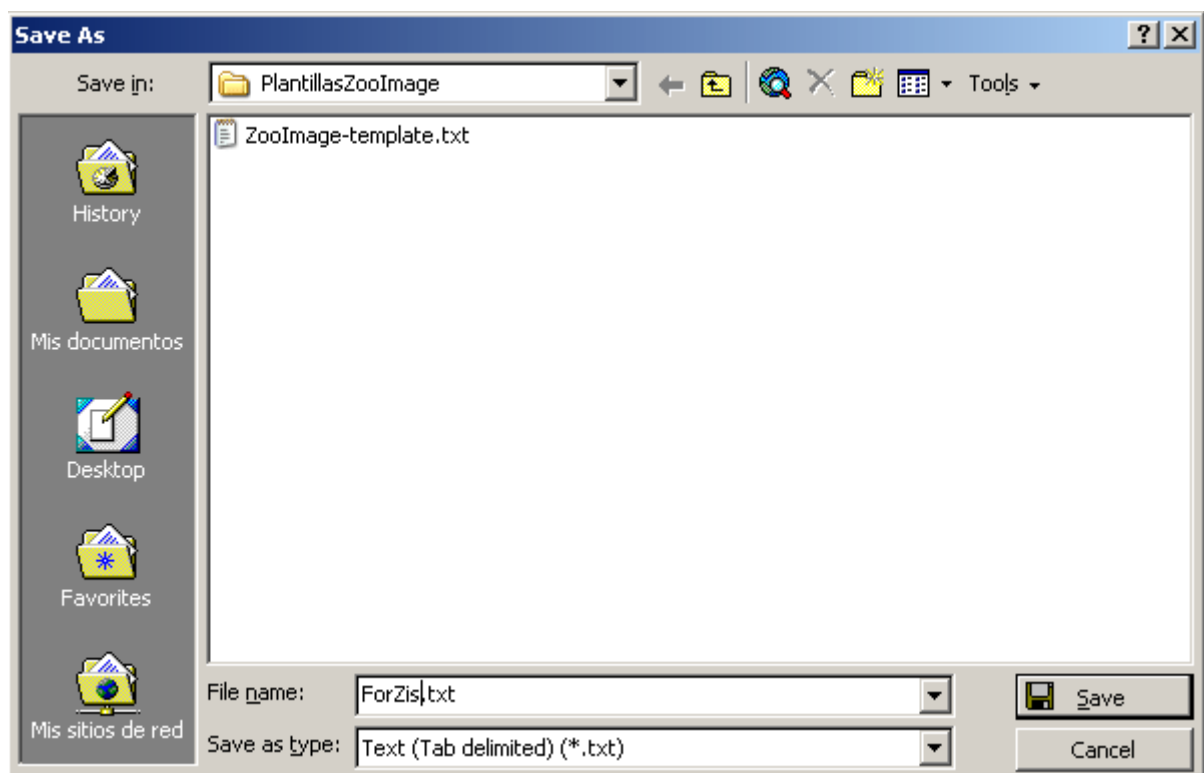
	A	B	C	D	E	F	G	H	I	J	K
1	Label	Code	SCS	Series	Cruise	Station	Date	Time	TimeZone	Latitude	Longitude
2	BIO.1998-5-18.P00 p-1	BIO	BIO1998	BIO	1	1998-05-18	10	10	3.9889	-43.475	
3	BIO.1998-5-18.P00 p-2	BIO	BIO1998	BIO	2	1998-05-18	10	10	3.9889	-43.5252	
4	BIO.1998-5-18.P00 p-3	BIO	BIO1998	BIO	3	1998-05-18	10	10	3.9889	-43.5747	
5	BIO.1998-5-18.P00 p-4	BIO	BIO1998	BIO	4	1998-05-18	10	10	3.9889	-43.6252	
6	BIO.1998-5-18.P00 p-5	BIO	BIO1998	BIO	5	1998-05-18	10	10	3.9889	-43.6758	
7	BIO.1998-5-18.P00 p-6	BIO	BIO1998	BIO	6	1998-05-18	10	10	3.9889	-43.7253	
8	BIO.1998-5-18.P00 p-7	BIO	BIO1998	BIO	7	1998-05-18	10	10	3.9889	-43.7752	
9	BIO.1998-5-18.P00 p-8	BIO	BIO1998	BIO	8	1998-05-18	10	10	3.9889	-43.8252	
10	BIO.1998-5-18.P00 p-9	BIO	BIO1998	BIO	9	1998-05-18	10	10	3.9889	-43.8764	
11	BIO.1998-5-18.P00 p-10	BIO	BIO1998	BIO	10	1998-05-18	10	10	3.9889	-43.9249	
12											

After completion for each sample, you must save it as a save it in plain text format. Select File → save as... menu entry.



	de	SCS	Series	Cruise	Station	Date	Time	TimeZone	Latitude	Longitude	
1											
2		BIO	BIO1998	BIO	1	1998-05-18	10	10	3.9889	-43.475	
3	BIO.1998-5-18.P00	p-2	BIO	BIO1998	BIO	2	1998-05-18	10	10	3.9889	-43.5252
4	BIO.1998-5-18.P00	p-3	BIO	BIO1998	BIO	3	1998-05-18	10	10	3.9889	-43.5747
5	BIO.1998-5-18.P00	p-4	BIO	BIO1998	BIO	4	1998-05-18	10	10	3.9889	-43.6252
6	BIO.1998-5-18.P00	p-5	BIO	BIO1998	BIO	5	1998-05-18	10	10	3.9889	-43.6758
7	BIO.1998-5-18.P00	p-6	BIO	BIO1998	BIO	6	1998-05-18	10	10	3.9889	-43.7253
8	BIO.1998-5-18.P00	p-7	BIO	BIO1998	BIO	7	1998-05-18	10	10	3.9889	-43.7752
9	BIO.1998-5-18.P00	p-8	BIO	BIO1998	BIO	8	1998-05-18	10	10	3.9889	-43.8252
10	BIO.1998-5-18.P00	p-9	BIO	BIO1998	BIO	9	1998-05-18	10	10	3.9889	-43.8764
11	BIO.1998-5-18.P00	p-10	BIO	BIO1998	BIO	10	1998-05-18	10	10	3.9889	-43.9249

In the Save as dialog box, change the type to ‘Text (Tab delimited) (*.txt)’.



Open the text file with notepad or another editor, select all the content (Ctrl + E or Ctrl + A) and copy it (Ctrl + C):



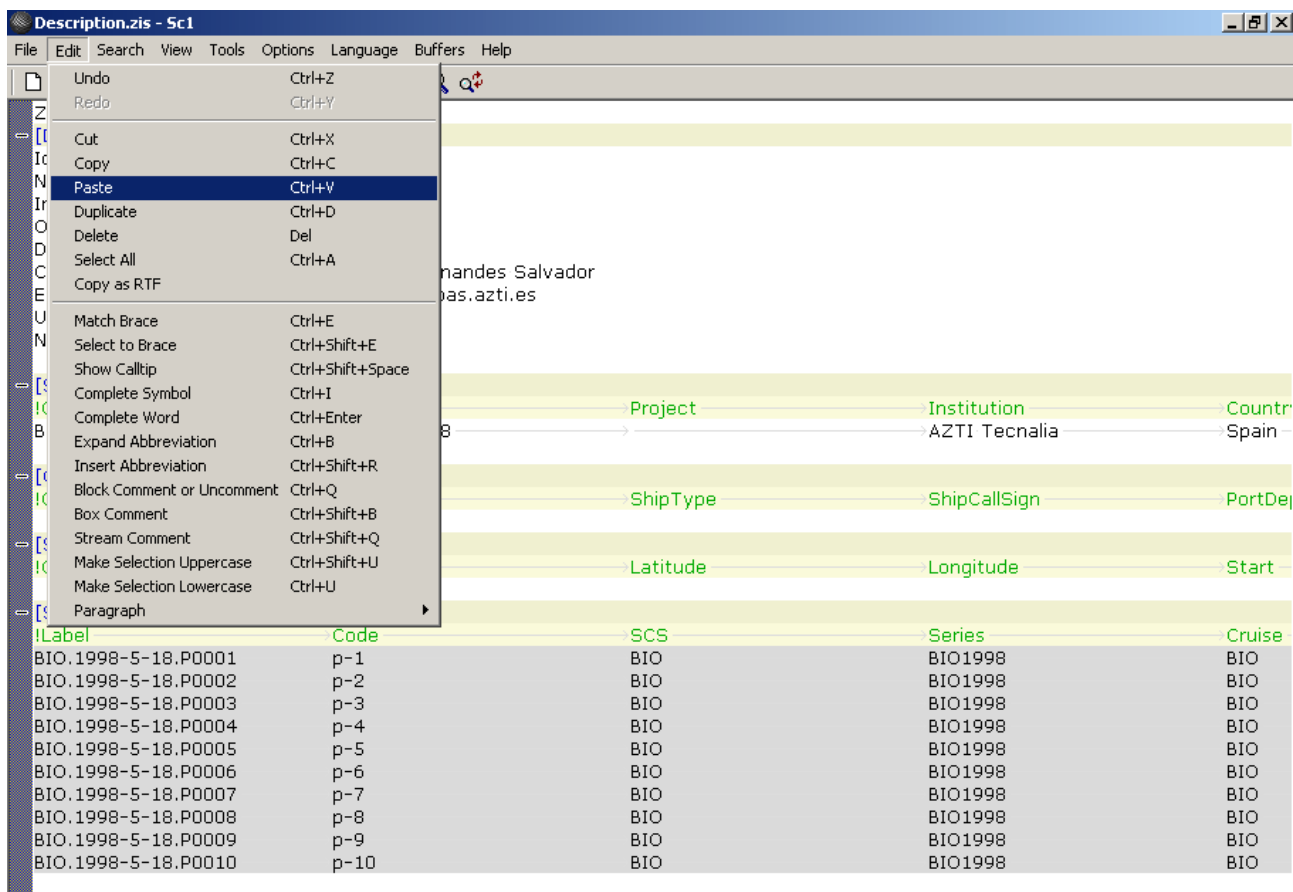
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ForZis.txt - Bloc de notas												
Archivo	Edición	Formato	Ver	Ayuda								
Label	Desahacer	Ctrl+Z			ries	Cruise	Station	Date	Time	TimeZone	Latitude	Longitude
Coords					GearType	Speed	weather	OpeningArea	MeshSize	Staining	DepthMin	DepthMax
SampVC	Cortar	Ctrl+X			ite			Preservative			Biovolume	Temperature
Salini	Copiar	Ctrl+C			1	BIO	BIO1998	BIO	1	1998-05-18	10	10
BIO.19	Pegar	Ctrl+V			220			220	10	50	8.120705227	1
-43.47	Eliminar	Supr			16.6			A				OBLICUO 2
BIO.19	Buscar...	Ctrl+B			2	BIO	BIO1998	BIO	2	1998-05-18	10	10
-43.52	Buscar siguiente	F3			220			50	10	9.073113347	1	OBLICUO 2
BIO.19	Reemplazar...	Ctrl+R			17			A				
-43.57	Ir a...	Ctrl+T			3	BIO	BIO1998	BIO	3	1998-05-18	10	10
BIO.19	Seleccionar todo	Ctrl+E			220			50	10	8.352472803	1	OBLICUO 2
-43.62	Hora y fecha	F5			16.2			A				
BIO.19					4	BIO	BIO1998	BIO	4	1998-05-18	10	10
-43.67584685					220			50	10	9.024977963	1	OBLICUO 2
4% buffered formalin					15.6			A				
BIO.1998-5-18.P0006					5	BIO	BIO1998	BIO	5	1998-05-18	10	10
-43.72534784					220			50	10	8.963259183	1	OBLICUO 2
4% buffered formalin					16			A				
BIO.1998-5-18.P0007					6	BIO	BIO1998	BIO	6	1998-05-18	10	10
-43.77518217					220			50	10	9.196144468	1	OBLICUO 2
4% buffered formalin					15.5			A				
BIO.1998-5-18.P0008					7	BIO	BIO1998	BIO	7	1998-05-18	10	10
-43.82518317					220			50	10	9.037138821	1	OBLICUO 2
4% buffered formalin					15.7			A				
BIO.1998-5-18.P0009					8	BIO	BIO1998	BIO	8	1998-05-18	10	10
-43.87635086					220			50	10	9.500978803	1	OBLICUO 2
4% buffered formalin					15.3			A				
BIO.1998-5-18.P0010					9	BIO	BIO1998	BIO	9	1998-05-18	10	10
-43.92485183					220			50	10	9.905132203	1	OBLICUO 2
4% buffered formalin					10			A				
BIO.1998-5-18.P0010					10	BIO	BIO1998	BIO	10	1998-05-18	10	10
-43.92485183					220			50	10	9.379065391	1	OBLICUO 2
4% buffered formalin					15.1			A				

Open the Description.zis that is with the templates file doing double click on the file:



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12.2.11 Process samples



12.2.12 View results



12.2.13 Export results

