How to setup and use the MAYDAY Affymetrix Processing pipeline (AffyPP)

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Preparations

Essential prerequisites

The MAYDAY Affymetrix Processing Pipeline (AffyPP) is part of the MAYDAY Processing Framework (MPF). As it includes an R-function to normalize the data, the R Interpreter with its dependencies and the BioConductor package has to be installed first. The statistical modules used by the pipeline require the `apache.commons.math` library. The `.jar`-file is available at the MAYDAY homepage `www.zbit.uni-tuebingen.de/pas/mayday/download/download.html`.

Installing R and the BioConductor package

R can be downloaded from `http://cran.r-project.org/`.
After installing R, the BioConductor package needs to be installed. In order to do so open your R command window and type the following:

```
source("http://bioconductor.org/biocLite.R")
biocLite()
```

1 Using the Affymetrix processing Pipeline (AffyPP)

1.1 Start the pipeline and select cel-files

To start the pipeline go to:

$$DataSet \rightarrow Importfromfile$$

A filechooser opens (see figure 1).

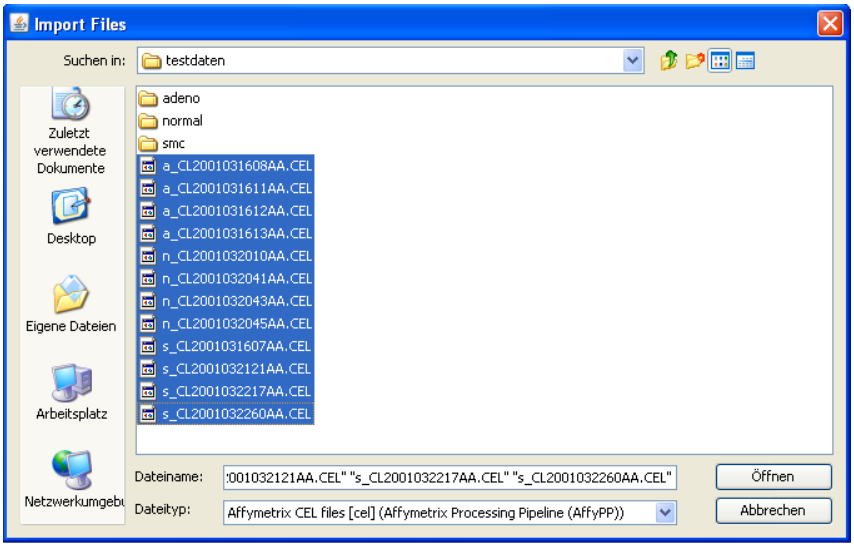


Figure 1: filechooser for selecting the cel-files

Select

"Affymetrix Cel files [cel] (Affymetrix Processing pipeline (AffyPP))"

as file type and then select all your cel-files and press *"OK"*.

1.2 Specify options for AffyPP

The MPF-Applicator opens (see figure 2) and you have to specify the following options:

1. **Files:** shows the number of files you have selected. Your selection can be changed by pressing "'browse"'
2. **Groups:** you don't have to specify any groups now. A class selection dialog will open after the normalization of the data for assigning groups
3. specify a name for the MIO groups which later contain the p-values. The individual MIO group names will be set to "user entered MIO name + ANOVA" for the ANOVA p-value and "user chosen MIO name + post-hoc t-test group n vs. m". The MIO group names are also important to for the p-value correction methods.

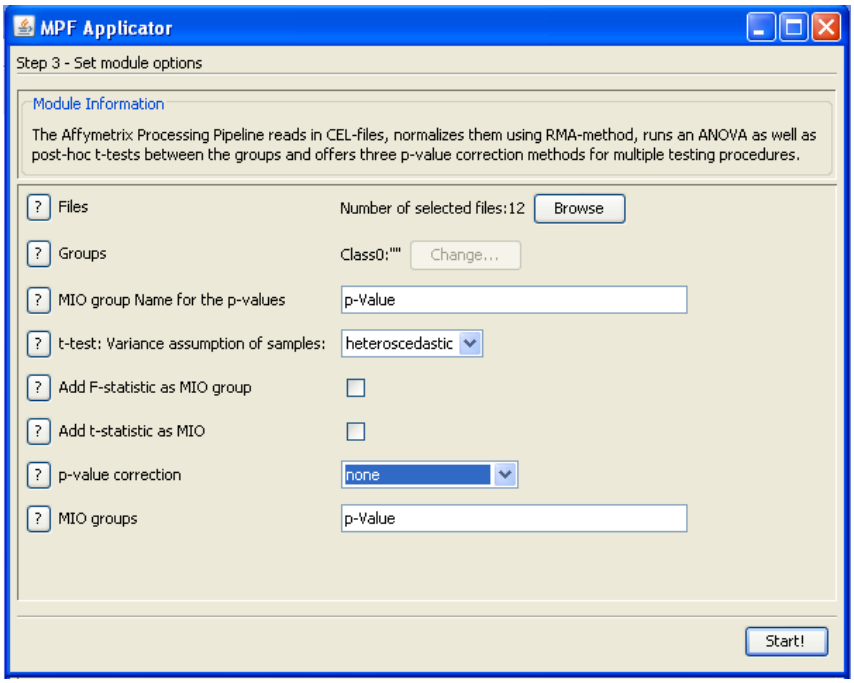


Figure 2: Specify options for AffyPP

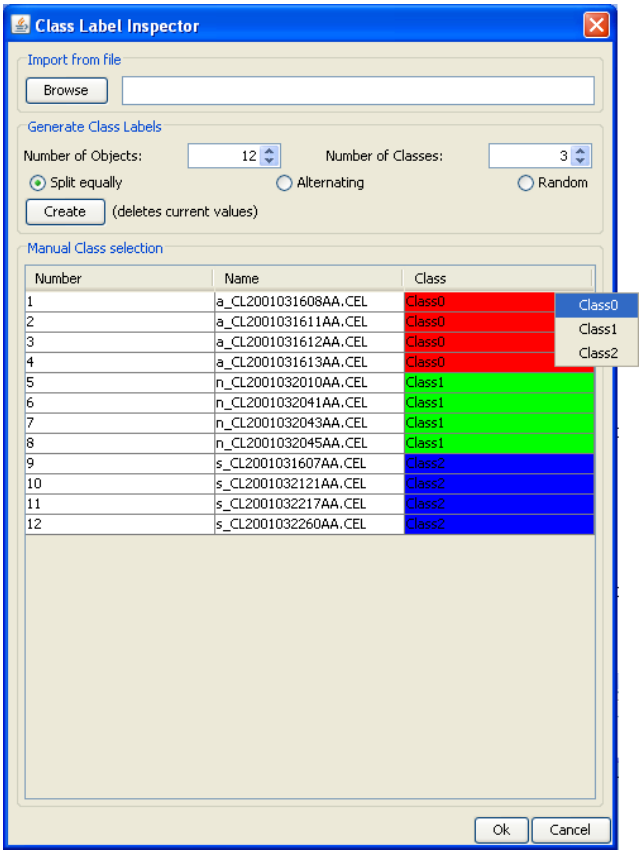
4. **t-test variance assumption of samples:** You have to choose whether you assume a heteroscedastic or homscedastic variance
5. select this if you want to have the F-statistic corresponding to the ANOVA p-value
6. select this if you want to have the t-statistic for every post-hoc t-test
7. select which p-value correction method you want to apply:
   - none
   - Bonferroni
   - Holm's Stepwise-Correction
   - False Discovery Rate (FDR)
8. enter the names of the MIO groups containing the p-values. If you can't remember them, leave it empty. The pipeline then opens a dialog, where all MIO groups are shown and you can select them easily.

In the example a heteroscedastic variance was assumed and no p-value correction was chosen. So the p-value correction method was set to *"none"*.

1.3 Assign classes

After the data is normalized a class selection dialog opens, where you can assign each cel-file to a class. To do so, choose the number of classes. The classes can

either be automaticly assigned (equally splitted or alternating) or manually by a right click on the class column and selection of the class. The example consists



of three classes. So the number of classes was set to *"3"*, and automaticly split (*"split equally"* → *"create"*) was chosen. The files were assigned as following (see figure 1.3):

class 1	**class 2**	**class 3**
a_CL2001031608AA.CEL	n_CL2001032010AA.CEL	s_CL2001031607AA.CEL
a_CL2001031611AA.CEL	n_CL2001032041AA.CEL	s_CL2001032121AA.CEL
a_CL2001031612AA.CEL	n_CL2001032043AA.CEL	s_CL2001032217AA.CEL
a_CL2001031613AA.CEL	n_CL2001032045AA.CEL	s_CL2001032260AA.CEL

1.4 Filter p-values

When the analysis is finished a MIO-Group-Selection dialog opens. Here you can select the p-values which should be used for filtering. The filter criteria is set to < 0.05. If multiple groups are selected, the criteria will be ALL p-values < 0.05. In

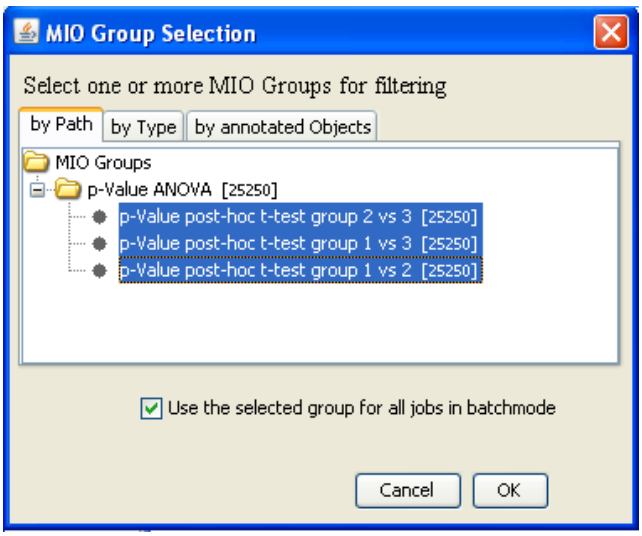


Figure 3: MIO Group Selection Dialog

the example all post-hoc t-test p-values (see figure 3) were chosen.

1.5 Set dataset properties and return data to MAYDAY

After the filtering is done a windows opens, were the dataset properties can be viewed and changed (see figure 4).

When pressing *"OK"* the dataset is returned to MAYDAY. A global probe list containing all probe sets (12625 probe sets) as well a filtered probe list (57 probe sets) containing all probe sets, which matched the criteria (here a p-value < 0.05 in ALL post-hoc t-tests) is returned to MAYDAY (see figure 5).

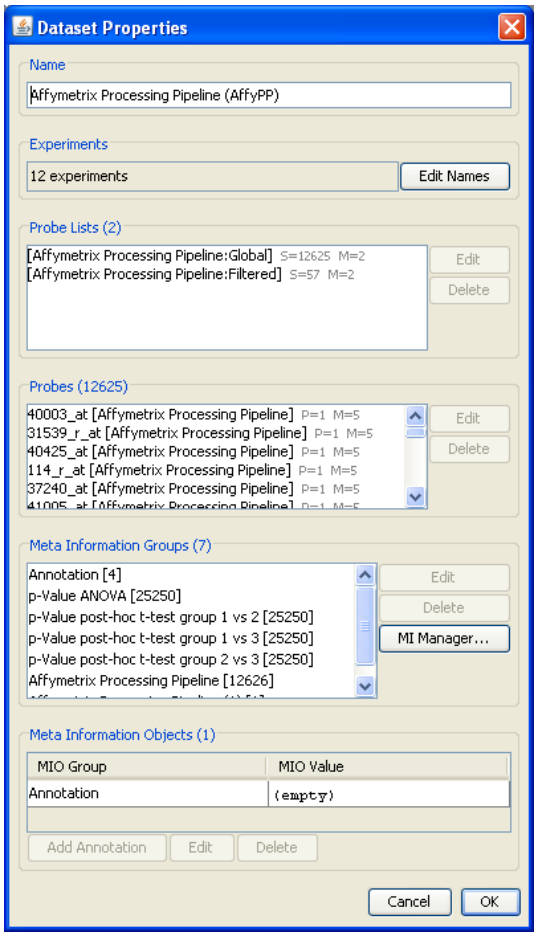


Figure 4: set dataset properties

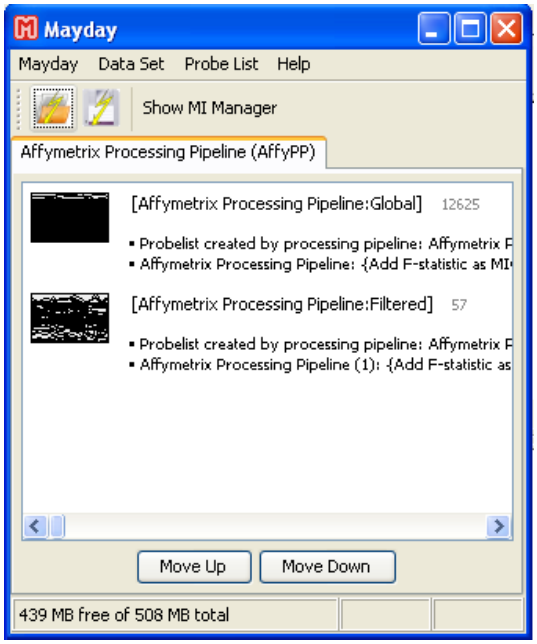


Figure 5: data returned to MAYDAY