**FlowFP procedure for microbiological datasets**

We recommend going line by line through the macro at the beginning to understand the individual steps and adapt them to your individual experiment. Later the complete procedure can be copied into a text document. The name has to be adapted to the ending .r (e.g. flowFP\_procedure.r). The macro can then be used for drag and drop into R.

#This script is written for analysis of cytometric histograms of complex microbial communities

#using the Bioconductor platform and the package flowFP.

#2013-02-12

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

biocLite("flowCore")

biocLite("flowFP")

#Install necessary Bioconductor packages.

#These steps have to be done only the first time.

library("flowCore")

library("flowFP")

#Load packages.

#These steps need to be done every time R is started.

fset <- read.flowSet(choose.files(), alter.names=TRUE, transformation=FALSE, column.pattern="[S4].Log")

#Load FCS files. A window is opened and the files can directly by chosen.

#This command works under windows only. Use “file.choose()“ under Linux.

#The dataset is named fset.

#"[S4].Log" defines the parameters that should be loaded, here FSC, SSC and FL4 in logarithmic scale.

#The more parameters are chosen the higher is the computational demand.

summary(fset)

#Summary of fset.

#The area of interest has to be defined. For that a threshold is set in the fluorescence channel and then applied to all samples in the fset.

#The threshold is flexible and depends on sample and staining.

#All events below this threshold are defined as noise or unstained cells.

Cells <- rectangleGate(filterId="Cells", list("FL.4.Log"=c(1000,Inf)))

Cells.subset <- Subset(fset, Cells)

xyplot(FL.4.Log ~ FS.Log, data=Cells.subset, colramp=colorRampPalette(c("white","grey","blue","green","yellow","red")))

#Visualization of the histograms.

model <- flowFPModel(Cells.subset[[1]], parameters=c("FL.4.Log", "FS.Log"), nRecursions=5)

#Defines a model on sample 1 using the parameters FL4.Log and FS.Log and 5 recursions.

#In alternative, the model can also be built from a number of samples (here sample 1, 2, and 5):

#model <- flowFPModel(Cells.subset[c(1,2,5)], parameters=c("FL.4.Log", "FS.Log"), nRecursions=5)

x11()

#opens a new plot

plot(model)

#The model is shown.

#Now, the model ist applied to all samples.

fp <- flowFP(Cells.subset, model)

#Different visualization options for the result are possible.

#For quality control the second option is the most convenient one.

x11()

plot(fp)

x11()

plot(fp, type="qc")

x11()

plot(fp, type="stack")

counts(fp)

#Displays the number of events per bin for all samples.