```r
# Module 2 code: Exploring FCM data in R
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# First, a short refresher to R
# Variable Assignment
x <- 5
y <- 10
x + y

# Vectors (one dimensional) and Matrices (two dimensional)
x <- c(1, 4, 5)
x
y <- seq(from = 1, to = 3, by = 1)
y
x + y

# Get help on how to construct a matrix
?matrix
A <- matrix(c(1, 2, 3,  11, 12, 13), nrow = 2, ncol = 3, byrow = TRUE)
A
rbind(x, y)
A + rbind(x, y)
A[1, 3]
A[2, ]
A[, 1]

# Lists and names
mylist <- list(`first` = x, `second` = y)
mylist
mylist[[1]]
mylist[["first"]]
mylist[[3]]
length(mylist)
length(x)
dim(A)

# More advanced functionality: which, intersect, union
?rnorm
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a <- rnorm(20)
a
which(a > 0)
a[1]
a[which(a > 0)]
which(a < -1)
intersect(which(a > 0), which(a < -1))
combined <- union(which(a > 0), which(a < -1))
combined
length(combined)

# Simple plotting
plot(a, col = "red")
plot(density(a))
hist(a)
plot(density(rnorm(1000)))
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# Flow Cytometry data
# Look at the help files to search for a function:
?read.FCS
??read.FCS
# Load the package which extends the functionality of R to work with flow
… data
library(flowCore)
# Make sure R knows which directory our data will be read from
getwd()
setwd('/home/rguru/Documents/Workshop/data')
dir()
dir('fullFCS/')
# Read an FCS file
f <- read.FCS('fullFCS/100715.fcs')
f
# 'f' is a flowFrame object. See ?flowFrame for details and to see what
… you can do with it
# how many events the file has
nrow(f)
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77 # the channel names:
78 colnames(f)
79 # Extract the expression values into a matrix
80 E <- exprs(f)
81 dim(E)
82 # The expression values are like a matrix -- each cell has a row of
   … measurements - one for each channel. Here are the first 10 cells:
83 E[1:10, ]
84 # Explore the meta data stored within the FCS file
85 f@description
86 names(f@description)
87 f@description$`TUBE NAME`
88 f@parameters@data
89 f@parameters@data[1, c("minRange", "maxRange")]
90
91 # Try a simple plot -- note the error R gives you. It says that you have
   … to first load the 'flowViz' library before you can plot FCM files.
92 plot(f, c("FSC-A", "SSC-A"))
93 library(flowViz)
94 plot(f, c("FSC-A", "SSC-A"), ylim = c(0, 5000), smooth=FALSE)
95 # Note SSC-A is the third parameter (P3) and the meta data tells us it is
   … to be viewed on a LOG scale:
96 colnames(f)[3] # See that this is SSC-A
97 f@description$`P3DISPLAY`
98
99 # Now read a flow set
100 fs <- read.flowSet(path = 'fullFCS', pattern = ".fcs")
101 fs
102 # You can see sample names as well as the channel names
103 sampleNames(fs)
104 length(fs)
105 colnames(fs)
106 # A flowSet object is similar to a list, a list of flowFrames
107 fs[["100715.fcs"]]
108 fs[[1]]
109
110 # Use fsApply to get cell counts for all samples
111 nrow(fs[[1]])
112 fsApply(fs, nrow)
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113 # Use fsApply to extract the TUBE NAME keyword in all samples
114 fsApply(fs, function(f) f@description$`TUBE NAME`)
115
116 ### Plotting excercise
    ##################################################
117 plot(fs[[2]], c("FSC-A", "SSC-A"), ylim = c(0, 5000), smooth = FALSE)
118 # Plot the density of the forward scatter area values for the first
    sample:
119 E <- exprs(fs[[1]])
120 fscValues <- E[, "FSC-A"]
121 fscValues[1:10]
122 plot(density(fscValues))
123
124 # We can plot all 3 samples on one plot:
125 par (mfrow = c(3, 1)) # This creates a plot region with a single column
    of 3 subplots
126 plot(fs[[1]], c("FSC-A", "SSC-A"), main = sampleNames(fs)[1], ylim = c(0,
    5000), smooth=FALSE)
127 plot(fs[[2]], c("FSC-A", "SSC-A"), main = sampleNames(fs)[1], ylim = c(0,
    5000), smooth=FALSE)
128 plot(fs[[3]], c("FSC-A", "SSC-A"), main = sampleNames(fs)[1], ylim = c(0,
    5000), smooth=FALSE)
129
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