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Module 2 -

Exploring FCM data in R

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Module 2: Exploring FCM data in R

Module Overview:

- Read in FCS files
- Explore data annotation and structure
- Visualize FCM data in R

R Refresher: a vector and a matrix

Note: some output is not shown to save space. Run the commands to see the output.

```r
# Variable Assignment
x ← 5
y ← 10
x + y

# Vectors (one dimensional)
x ← c(1, 4, 5)
y ← seq(from = 1, to = 3, by = 1)
y
x + y

# Get help on how to construct a matrix (two dimensional)
?matrix
A ← matrix(c(1, 2, 3,  11, 12, 13), nrow = 2, ncol = 3,
    byrow = TRUE)
A
rbind(x, y)
A + rbind(x, y) # see ?rbind for explanation
A[1, 3] # Access entry in first row, third column
A[2, ] # Access all entries in second row
A[, 1] # All entries in first column
```

R Refresher: A list with named entries

```
# Lists and names
mylist ← list(‘first‘ = x, ‘second‘ = y)

# Extract the first entry in the list by index (1) or name ("first"):
mylist[[1]]
[1] 1 4 5
mylist[["first"]]
[1] 1 4 5

# Checking the size of objects
# Vectors and lists have ’length’s, matrices have ’dim’ensions
length(mylist)
[1] 2
length(x)
[1] 3
dim(A)
[1] 2 3
length(A)
[1] 6
```

R Refresher: which, intersect, union

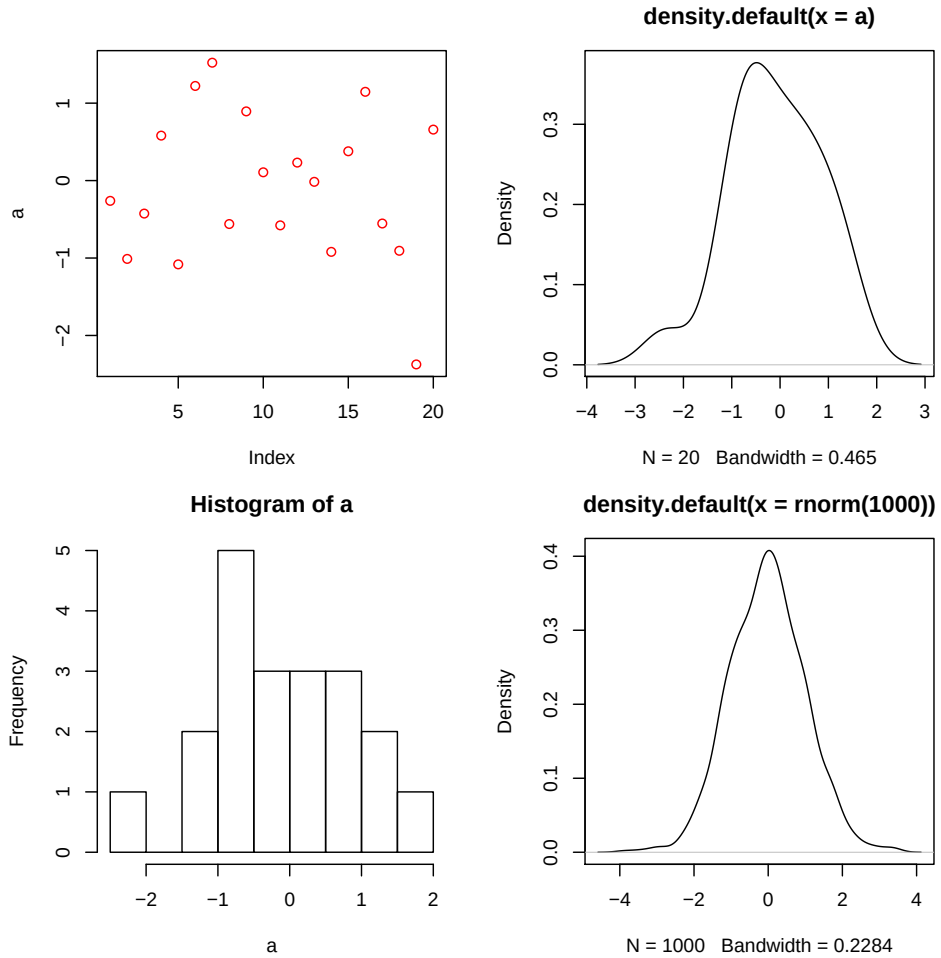
```
# Generate 20 random numbers from a normal distribution
?rnorm
a ← rnorm(20)
a
```

```
[1]  0.16215786  0.42892603 -0.40585986 -0.41403764  1.08375740 -0.16635696 -0.11281127
-1.97296631 -1.43725793 -0.61669020 0.92111531 -1.76906195 -0.23469280 -0.28725082
-1.68470666 0.86727154  1.75077450  0.08697872 -1.47718350 -0.03710011
```

```
# Identify the indices of 'a' > 0, then values < -1
which(a > 0)
[1]  1  2  5 11 16 17 18
which(a < -1)
[1]  8  9 12 15 19

# Check that their intersection is empty, take union
intersect(which(a > 0), which(a < -1))
[1] integer(0) # empty intersection!
combined ← union(which(a > 0), which(a < -1))
combined
[1]  1  2  5 11 16 17 18  8  9 12 15 19
```

R Refresher: dot, density, histogram plot




```
# Simple plotting functionality
plot(a, col = "red")
plot(density(a))
hist(a)
plot(density(rnorm(1000)))
```

FCM data: reading FCS files in R

```
# Load the package for reading FCM data
library(flowCore)
# Make sure R knows which directory our data is in
setwd('/home/rguru/Documents/Workshop/data')
f ← read.FCS('fullFCS/100715.fcs')
f
```

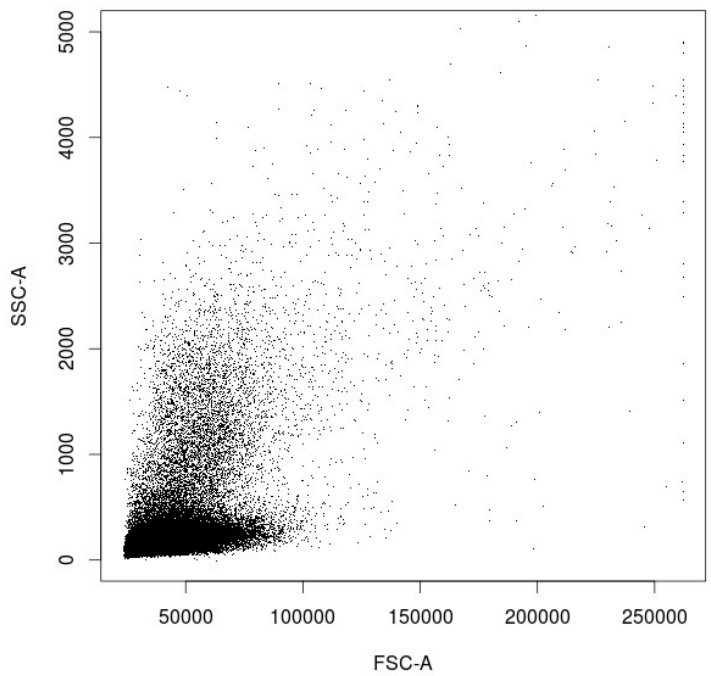
```
flowFrame object '0d8e743a-05fe-4e8b-9ec4-25993c124ee2'
with 65016 cells and 16 observables:
       name          desc  range     minRange maxRange
$P1   FSC-A              262207 23406.000000   262206
$P2   FSC-H              262207 27008.500000   262206
$P3   SSC-A              261588    -8.014621   261587
$P4  B515-A         KI67 261588   -67.282539   261587
$P5  R780-A          CD3 261588   -67.119034   261587
$P6  R710-A         CD28 261588   -44.558552   261587
$P7  R660-A       CD45RO 261588   -79.819801   261587
$P8  V800-A          CD8 261588  -110.409325   261587
$P9  V655-A          CD4 261588   -66.276711   261587
$P10 V585-A         CD57 261588  -110.472748   261587
$P11 V450-A VIVID / CD14 261588   -28.876564   261587
$P12 G780-A         CCR5 261588  -110.527817   261587
$P13 G710-A         CD19 261588   -89.503387   261587
$P14 G660-A         CD27 261588   -51.957710   261587
$P15 G610-A         CCR7 261588   -61.939350   261587
$P16 G560-A        CD127 261588   -33.256630   261587
253 keywords are stored in the 'description' slot
```

FCM data: exploring FCS meta data

```
# 'f' is a flowFrame object which comes with meta data.
# The channel labels:
colnames(f)
# The expression values measured for each cell:
E ← exprs(f)
dim(E)
# Look at first 10 rows (cells) of E
E[1:10, ]

# Study all the 'keyword' information stored in the file
f@description
# Access each 'keyword' one at a time
f@description$`TUBE NAME`
# Access parameter information such as range of expression
    values of FSC-A:
f@parameters@data
f@parameters@data[1, c("minRange", "maxRange")]
    minRange maxRange
$P1    23406   262206
```

Plotting FCM data in R




```
# First load the library which helps plot FCM data
library(flowViz)
plot(f, c("FSC-A", "SSC-A"), ylim = c(0,5000), smooth=FALSE)
# Note SSC-A is the third parameter (P3) and the meta data
    tells us it is to be viewed on a LOG scale:
colnames(f)[3]
[1] "SSC-A"
f@description$‘P3DISPLAY‘
[1] "LOG"
```

Reading multiple FCS files

```
# 'f' above is a flowFrame object.
# A set of flowFrame objects is a flowSet object:
fs ← read.flowSet(path = 'fullFCS', pattern = ".fcs")
fs
A flowSet with 3 experiments.
  column names:
  FSC-A FSC-H SSC-A B515-A R780-A R710-A R660-A V800-A V655-
      A V585-A V450-A G780-A  G710-A G660-A G610-A G560-A

# Explore the meta data for the flowSet:
sampleNames(fs)
[1] "100715.fcs" "109567.fcs" "113548.fcs"
length(fs)
[1] 3

# A flowSet object is closely related to a list
# You can access the first frame by index or name
fs[["100715.fcs"]]
fs[[1]]
```

Using 'fsApply'

```
# Use fsApply to get cell counts for all samples
nrow(fs[[1]])
[1] 65016
fsApply(fs, nrow)
               [,1]
100715.fcs  65016
109567.fcs 160074
113548.fcs 177102

# Use 'fsApply' to extract the TUBE NAME keyword
# This is useful in identifying control and stained, or
    stimulated and unstimulated samples in large data sets
fsApply(fs, function(f) f@description$`TUBE NAME`)
             [,1]
100715.fcs "Tube_025"
109567.fcs "Tube_017"
113548.fcs "Tube_003"
```

LUNCH 1hr