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title: "Exploring FCM Data In R"
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date: "7/11/2017"
output:
  html_document:
    toc: yes
  html_notebook:
    toc: yes
  pdf_document:
    highlights: tango
    keep_tex: yes
    number_sections: yes
    toc: yes
---

LICENSE


```{r setup, include=FALSE}
knitr::opts_chunk$set(echo = FALSE)
```

Flow Cytometry data

Look at the help files to search for a function:

```{r}
?read.FCS
```


Load the package which extends the functionality of R to work with flow data

```{r}
library(flowCore)
```

Make sure R knows which directory our data will be read from

```{r}
getwd()
setwd('./data')
dir()
dir('fullFCS/')
```
```

````
```

## Read an FCS file

```{r}
f <- read.FCS('./data/fullFCS/100715.fcs')
f
# 'f' is a flowFrame object. See ?flowFrame for details and to see what you can do with it
```

## Get some information about the FCS file

How many events the file has

```{r}
nrow(f)
```

The channel names:

```{r}
colnames(f)
```

Extract the expression values into a matrix

```{r}
E <- exprs(f)
```

What are teh dimentions of the data?

```{r}
dim(E)
```

## The expression values are like a matrix

-- Each cell has a row of measurements - one for each channel. Here are the first 10 cells:

```{R}
E[1:10, ]
```

## Explore the meta data stored within the FCS file
````
````

````
 91
 92 ```{r}
 93 f@description
 94 ```
 95
 96 ```{r}
 97 names(f@description)
 98 ```
 99
100 ```{r}
101 f@description$`TUBE NAME`
102 ```
103
104 ```{r}
105 f@parameters@data
106 ```
107
108 ```{r}
109 f@parameters@data[1, c("minRange", "maxRange")]
110 ```
111
112 ## Try a simple plot
113
114 -- note the error R gives you.
115 It says that you have to first load the 'flowViz' library
    before you can plot FCM files.
116
117 ```{r}
118 plot(f, c("FSC-A", "SSC-A"))
119 ```
120
121 ```{r}
122 library(flowViz)
123 plot(f, c("FSC-A", "SSC-A"), ylim = c(0, 5000), smooth=FALSE)
124 ```
125
126 ```{r}
127 # Note SSC-A is the third parameter (P3) and the meta data
    tells us it is to be viewed on a LOG scale:
128 colnames(f)[3] # See that this is SSC-A
129 f@description$`P3DISPLAY`
130 ```
131
132 ## Read a flow set
133
134 ```{r}
135 # Now read a flow set
````

```
fs <- read.flowSet(path = './data/fullFCS', pattern = ".fcs")
fs
# You can see sample names as well as the channel names
sampleNames(fs)
length(fs)
colnames(fs)
# A flowSet object is similar to a list, a list of flowFrames
fs[["100715.fcs"]]
fs[[1]]
```

fsApply

```{r}
# Use fsApply to get cell counts for all samples
nrow(fs[[1]])
fsApply(fs, nrow)
# Use fsApply to extract the TUBE NAME keyword in all samples
fsApply(fs, function(f) f@description$`TUBE NAME`)
```

Plotting excercise

```{r}
### Plotting excercise
    ######################################################
plot(fs[[2]], c("FSC-A", "SSC-A"), ylim = c(0, 5000), smooth
    = FALSE)
```

```{r}
# Plot the density of the forward scatter area values for the
    first sample:
E <- exprs(fs[[1]])
fscValues <- E[, "FSC-A"]
fscValues[1:10]
plot(density(fscValues))
```

plot all 3 samples on one plot

```{r}
# We can plot all 3 samples on one plot:
par (mfrow = c(3, 1)) # This creates a plot region with a
   single column of 3 subplots
plot(fs[[1]], c("FSC-A", "SSC-A"), main = sampleNames(fs)[1
   ], ylim = c(0, 5000), smooth=FALSE)
```

```
178 plot(fs[[2]], c("FSC-A", "SSC-A"), main = sampleNames(fs)[1
    ], ylim = c(0, 5000), smooth=FALSE)
179 plot(fs[[3]], c("FSC-A", "SSC-A"), main = sampleNames(fs)[1
    ], ylim = c(0, 5000), smooth=FALSE)
180 ```
```