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title: "Exploring FCM Data In R"
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LICENSE


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

## R Markdown

This is an R Markdown presentation. Markdown is a simple
formatting syntax for authoring HTML, PDF, and MS Word
documents. For more details on using R Markdown see
<http://rmarkdown.rstudio.com>.

When you click the **Knit** button a document will be generated
that includes both content as well as the output of any
embedded R code chunks within the document.


############################################################

# Flow Cytometry data

## Look at the help files to search for a function:

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

Slide

```{r}
# how many events the file has
nrow(f)
# the channel names:
colnames(f)
# Extract the expression values into a matrix
E <- exprs(f)
```

```{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

```{r}
f@description
names(f@description)
f@description$`TUBE NAME`
f@parameters@data
f@parameters@data[1, c("minRange", "maxRange")]
```

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.

```{r}
plot(f, c("FSC-A", "SSC-A"))
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] # See that this is SSC-A
f@description$`P3DISPLAY`
```

Read a flow set

```{r}
# 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)
# 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)
plot(fs[[2]], c("FSC-A", "SSC-A"), main = sampleNames(fs)[1],
ylim = c(0, 5000), smooth=FALSE)
plot(fs[[3]], c("FSC-A", "SSC-A"), main = sampleNames(fs)[1],
ylim = c(0, 5000), smooth=FALSE)
```