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title: "Exploring FCM Data In R"
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output:
  html_document:
    toc: yes
  html_notebook:
    toc: yes
  pdf_document:
    highlights: tango
    keep_tex: yes
    number_sections: yes
    toc: yes
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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

```{r}
f@description
```

```{r}
names(f@description)
```

```{r}
f@description$`TUBE NAME`
```

```{r}
f@parameters@data
```

```{r}
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"))
```

```{r}
library(flowViz)
plot(f, c("FSC-A", "SSC-A"), ylim = c(0, 5000), smooth=FALSE)
```

```{r}
# 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)
```

```{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)
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)
```