R for cytometry - fcs files

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fcs file structures

- `flowFrame`: Represents data contained in FCS files. There are three parts:
  1. raw measurements
  2. annotation for the parameters
  3. additional annotation
- `flowSet`: Container of several `flowFrames`.

reading fcs files

```
require(flowCore)
# a sample file
fcsFile <- system.file("extdata", "0877408774.B08",
                       package="flowCore")

# read fcs file
ff <- read.FCS(fcsFile)
```

looking at fcs files

```
# annotation
pData(ff@parameters)
```

```
##       name                  desc range minRange maxRange
## $P1 FSC-H                FSC-H  1024        0     1023
## $P2 SSC-H                SSC-H  1024        0     1023
## $P3 FL1-H                 <NA>  1024        1    10000
## $P4 FL2-H                 <NA>  1024        1    10000
## $P5 FL3-H                 <NA>  1024        1    10000
## $P6 FL1-A                 <NA>  1024        0     1023
## $P7 FL4-H                 <NA>  1024        1    10000
## $P8  Time Time (51.20 sec.)  1024        0     1023
```

looking at fcs files

```
# look at data
ff@exprs[1:10, 1:5]
```

```
##        FSC-H SSC-H      FL1-H      FL2-H     FL3-H
##   [1,]   382    77 259.455272   1.000000  7.566695
##   [2,]   628   280   9.057978  48.260715 10.273508
##   [3,]  1023   735 537.611747  56.234133  6.915821
##   [4,]   373   128   6.152654  24.144182  2.329097
##   [5,]  1023  1023 259.455272 791.475544 39.241898
##   [6,]   489   292   5.002865  28.902639  3.995421
##   [7,]   869   453 552.315842   5.935229  4.958068
##   [8,]   422   136 228.757320   8.896491  2.072079
##   [9,]   430   131 184.342299   5.473703  4.958068
##  [10,]   364   129 220.673407   5.935229  1.910953
```

Writing fcs files

```
# specify output location and name
setwd("xxx")
data_dir <- "../data/"
file_name <- "test_file.fcs"
# write fcs file
write.FCS(ff,
          filename = paste0(data_dir, file_name))
```

Important: You are responsible that the expressions and the metadata in the new fcs-file correspond to each other!

reading multiple fcs files

```
# file location
fcs.loc <- system.file("extdata",
                       package="flowCore")
files <- paste(fcs.loc, dir(fcs.loc),
               sep="/")[1:3]

# read fcs files
fset <- read.flowSet(files)
```

looking at flowSet

```
# flowSet
fset
```

```
## A flowSet with 3 experiments.
##
##   column names:
##   FSC-H SSC-H FL1-H FL2-H FL3-H FL1-A FL4-H Time
```

```
# flowFrame
fset[[1]]@exprs[1:3, 1:5]
```

```
##       FSC-H SSC-H      FL1-H    FL2-H     FL3-H
## [1,]    382    77 259.455272  1.00000  7.566695
## [2,]    628   280   9.057978 48.26071 10.273508
## [3,]   1023   735 537.611747 56.23413  6.915821
```

Data from cytobank

```
# load CRAN package
require(CytobankAPI)
# Authenticate via authentication token
cyto_session <- authenticate(site="cellmass",
                             auth_token="xxx")
# specify experiment id
experiment_id <- 123
# specify data directory
data_dir <- "fcs_from_cytobank"
dir.create(data_dir, recursive = TRUE)
# list fcs files
files <- fcs_files.list(cyto_session,
                        experiment_id = experiment_id)
```

Data from cytobank

```
# download fcs files
downloaded_zip <- fcs_files.download_zip(
  UserSession = cyto_session,
  experiment_id = experiment_id,
  fcs_files = files[, "id"],
  directory = data_dir,
  timeout = 60*nrow(files))
unzip(downloaded_zip, exdir = data_dir)
# read fcs files
fset <- read.flowSet(paste0(data_dir, files))
```