R for cytometry - FlowSOM

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Download 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 <- 12
# 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)
```

Download 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

```
require(flowCore)
# read fcs file
files <- paste0(data_dir, "/",
                list.files(data_dir,
                           pattern = ".fcs"))
fset <- read.flowSet(files)
# decide on lineage markers
pData(fset[[1]]@parameters)
lineage_markers <- c(
  "In(114.903)-Dual", "Cd(109.903)-Dual",
  "Nd(143.910)-Dual", "Nd(144.912)-Dual",
  "Nd(145.913)-Dual", "Nd(147.916)-Dual",
  "Sm(146.914)-Dual", "Er(166.932)-Dual")
```

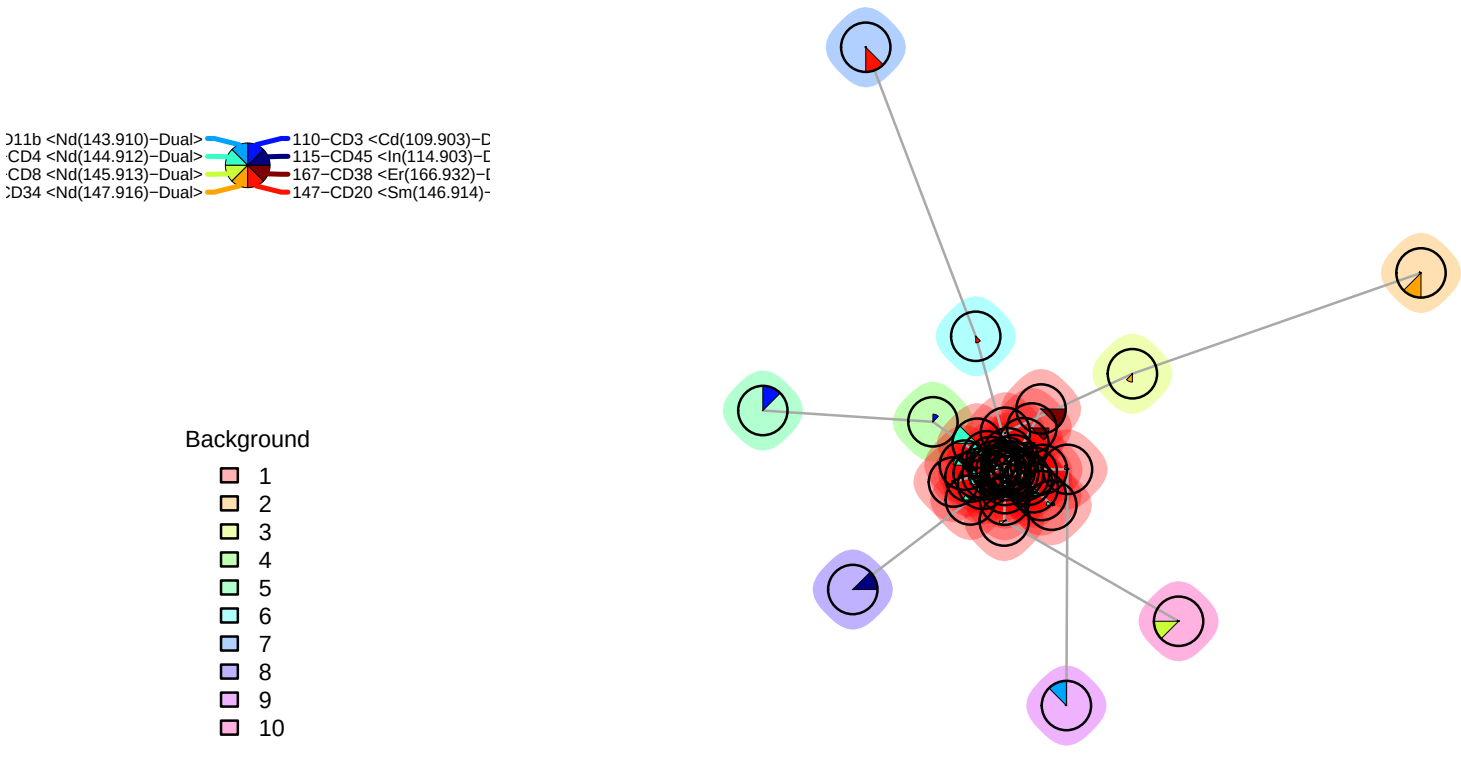
FlowSOM

```
require(FlowSOM)
# Set SOM parameters
flowSOM_metaClusters <- 10
flowSOM_xdim <- 7
flowSOM_ydim <- 7
flowSOM_seed <- 20180216 # for reproducible results

# run self-organizing maps
fsom <- FlowSOM(fset,
                colsToUse = lineage_markers,
                xdim=flowSOM_xdim,
                ydim=flowSOM_xdim,
                nClus = flowSOM_metaClusters,
                seed = flowSOM_seed)
```

Plotting with FlowSOM

```
PlotStars(UpdateNodeSize(fsom[[1]], reset=T),
          view = "MST",
          backgroundValues = as.factor(fsom[[2]]))
```



Summarize results

```
fset_new <- fset
# get clusters
clusters <- fsom$FlowSOM$map$mapping[, 1]
meta_clusters <- as.numeric(fsom$metaclustering[clusters])
filenames <- rep(sampleNames(fset),
                 fsApply(fset, nrow, use.exprs = TRUE))
res_data <- data.frame(clusters = clusters,
                       meta_clusters = meta_clusters,
                       filenames = filenames)
head(res_data)
```

```
##   clusters meta_clusters              filenames
## 1       28             1 Marrow1_01_Basal1.fcs
## 2       20             1 Marrow1_01_Basal1.fcs
## 3        1             1 Marrow1_01_Basal1.fcs
## 4       33             1 Marrow1_01_Basal1.fcs
## 5        1             1 Marrow1_01_Basal1.fcs
## 6       43             1 Marrow1_01_Basal1.fcs
```

Update data

```
update_ff <- function(ff, res_data){
  rd <- res_data[res_data$filenames == identifier(ff),
                 c("clusters", "meta_clusters")]
  ff@exprs <- cbind(ff@exprs, as.matrix(rd))
  ff@parameters@data <- rbind(
    ff@parameters@data,
    data.frame(
      name = c("cluster", "meta_cluster"),
      desc = "som_clustering",
      range = c(max(res_data$clusters),
                max(res_data$meta_clusters)),
      minRange = 1,
      maxRange = c(max(res_data$clusters),
                   max(res_data$meta_clusters)),
      row.names =
        paste0("$P", nrow(ff@parameters@data) + 1:2)))
  ff
}
```

Update data

```
# change data
fset_new <- fsApply(fset, update_ff,
                    res_data = res_data)
fset_new
```

```
## A flowSet with 3 experiments.
##
##   column names:
##   Time Cell_length Ir(190.960)-Dual Ir(192.962)-Dual Rh
```

Writing FCS file

```
# write file
new_files <- gsub(".fcs", "_som.fcs", files)
flowCore::write.flowSet(fset_new,
                        outdir = ".",
                        filename = new_files)
```

```
## [1] "."
```

```
# zip output files
zip_filename <- paste0(data_dir, "clustered_som.zip")
zip(zip_filename, new_files)
```

Uploading files to cytobank

```
# create new experiment
new_experiment_name <- "test_som"
new_experiment <- experiments.new(
  cyto_session,
  experiment_name = new_experiment_name,
  purpose = "testing som")

# upload files
fcs_files.upload_zip(cyto_session,
                     experiment_id = new_experiment$id,
                     file_path = zip_filename,
                     timeout = 720)
```