Flow Cytometry data analysis workflow

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IntroduCtion

In this workflow I will jump straight ahead to future profiling flavoursomeness data. First, I will do clustering and differential analysis and later, I will discuss data plot plotting strategies.

Part 1: Clustering and differential Analysis

```
BiocManager::install("CytoNorm")
devtools::install_github("JinmiaoChenLab/Rphenograph")
devtools::install_github("JinmiaoChenLab/RchyOptimyx")
devtools::install_github("JinmiaoChenLab/flowPeak")
devtools::install_github("JinmiaoChenLab/Rtsne.multicore")
devtools::install_github("JinmiaoChenLab/ddpcr")
devtools::install_github("JinmiaoChenLab/flowClean")
devtools::install_github("JinmiaoChenLab/flowAI")
devtools::install_github("JinmiaoChenLab/flowFP")
devtools::install_github("JinmiaoChenLab/Rclusterplus")
devtools::install_github("JinmiaoChenLab/flowCut")
devtools::install_github("JinmiaoChenLab/flowMeans")
devtools::install_github("JinmiaoChenLab/flowMerge")
devtools::install_github("JinmiaoChenLab/cytofkit")
devtools::install_github("JinmiaoChenLab/Rtnse")
devtools::install_github("JinmiaoChenLab/DEPtest")
devtools::install_github("JinmiaoChenLab/ddpcrquant.v1d0")
devtools::install_github("JinmiaoChenLab/Rtsne.multicore")
devtools::install_github("JinmiaoChenLab/RphenoVertex")
devtools::install_github("JinmiaoChenLab/cytofWorkflow")
devtools::install_github("JinmiaoChenLab/
```

```
> plot_grid(plot_legend, ncol = 1)
Warning messages:
1: Ignoring unknown parameters: fill
2: Ignoring unknown parameters: fill
> dev.off()
pdf
  2
```

```
> dev.off()
pdf
  2
```

Part 2: Filtering and gating workflow

```
# Filtering Parameters for CTRL_Tube_001_001.fcs
# Version: 1.0
# Date: 2025-10-17
# Author: Jacob Rozmus
# Description:
# Initial gate before clean
# Manual gate
# Initial exploration of the file
# Automated gate
# Output:
# gating_ellipse_1.csv: Coordinates
# ROI(s) visualized: Summary
# Notes: None
# Dependencies: None

# Dependencies for gating
# Ensure the gating_ellipse_1.csv exists in the current directory.
# If not, run the initial gate creation script first.
ellipse_1 <- read.csv("gating_ellipse_1.csv")
ellipse_1_matrix <- as.matrix(ellipse_1[, c("x", "y")])

# Apply the gate to the data using the ellipse matrix
fr_ellipse <- Rectangle(ellipse = ellipse_1_matrix)
fr_gated <- Subset(fr, fr_ellipse)

print(paste("Original events: ", nrow(exprs(fr)), sep=""))
## [1] "Original events: 1000000"
print(paste("Gated events: ", nrow(exprs(fr_gated)), sep=""))
## [1] "Gated events: 1000000"

# Apply gating to the other files
fr_002 <- read.FCS("CTRL_Tube_001_002.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_002_gated <- Subset(fr_002, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_002_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_003 <- read.FCS("CTRL_Tube_001_003.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_003_gated <- Subset(fr_003, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_003_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_004 <- read.FCS("CTRL_Tube_001_004.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_004_gated <- Subset(fr_004, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_004_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_005 <- read.FCS("CTRL_Tube_001_005.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_005_gated <- Subset(fr_005, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_005_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_006 <- read.FCS("CTRL_Tube_001_006.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_006_gated <- Subset(fr_006, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_006_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_007 <- read.FCS("CTRL_Tube_001_007.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_007_gated <- Subset(fr_007, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_007_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_008 <- read.FCS("CTRL_Tube_001_008.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_008_gated <- Subset(fr_008, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_008_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_009 <- read.FCS("CTRL_Tube_001_009.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_009_gated <- Subset(fr_009, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_009_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_010 <- read.FCS("CTRL_Tube_001_010.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_010_gated <- Subset(fr_010, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_010_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_011 <- read.FCS("CTRL_Tube_001_011.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_011_gated <- Subset(fr_011, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_011_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_012 <- read.FCS("CTRL_Tube_001_012.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_012_gated <- Subset(fr_012, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_012_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_013 <- read.FCS("CTRL_Tube_001_013.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_013_gated <- Subset(fr_013, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_013_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_014 <- read.FCS("CTRL_Tube_001_014.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_014_gated <- Subset(fr_014, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_014_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_015 <- read.FCS("CTRL_Tube_001_015.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_015_gated <- Subset(fr_015, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_015_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_016 <- read.FCS("CTRL_Tube_001_016.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_016_gated <- Subset(fr_016, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_016_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_017 <- read.FCS("CTRL_Tube_001_017.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_017_gated <- Subset(fr_017, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_017_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_018 <- read.FCS("CTRL_Tube_001_018.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_018_gated <- Subset(fr_018, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_018_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_019 <- read.FCS("CTRL_Tube_001_019.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_019_gated <- Subset(fr_019, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_019_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_020 <- read.FCS("CTRL_Tube_001_020.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_020_gated <- Subset(fr_020, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_020_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_021 <- read.FCS("CTRL_Tube_001_021.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_021_gated <- Subset(fr_021, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_021_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_022 <- read.FCS("CTRL_Tube_001_022.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_022_gated <- Subset(fr_022, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_022_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_023 <- read.FCS("CTRL_Tube_001_023.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_023_gated <- Subset(fr_023, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_023_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_024 <- read.FCS("CTRL_Tube_001_024.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_024_gated <- Subset(fr_024, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_024_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_025 <- read.FCS("CTRL_Tube_001_025.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_025_gated <- Subset(fr_025, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_025_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_026 <- read.FCS("CTRL_Tube_001_026.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_026_gated <- Subset(fr_026, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_026_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_027 <- read.FCS("CTRL_Tube_001_027.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_027_gated <- Subset(fr_027, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_027_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_028 <- read.FCS("CTRL_Tube_001_028.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_028_gated <- Subset(fr_028, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_028_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_029 <- read.FCS("CTRL_Tube_001_029.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_029_gated <- Subset(fr_029, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_029_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_030 <- read.FCS("CTRL_Tube_001_030.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_030_gated <- Subset(fr_030, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_030_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_031 <- read.FCS("CTRL_Tube_001_031.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_031_gated <- Subset(fr_031, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_031_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_032 <- read.FCS("CTRL_Tube_001_032.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_032_gated <- Subset(fr_032, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_032_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_033 <- read.FCS("CTRL_Tube_001_033.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_033_gated <- Subset(fr_033, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_033_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_034 <- read.FCS("CTRL_Tube_001_034.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_034_gated <- Subset(fr_034, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_034_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_035 <- read.FCS("CTRL_Tube_001_035.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_035_gated <- Subset(fr_035, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_035_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_036 <- read.FCS("CTRL_Tube_001_036.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_036_gated <- Subset(fr_036, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_036_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_037 <- read.FCS("CTRL_Tube_001_037.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_037_gated <- Subset(fr_037, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_037_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_038 <- read.FCS("CTRL_Tube_001_038.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_038_gated <- Subset(fr_038, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_038_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_039 <- read.FCS("CTRL_Tube_001_039.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_039_gated <- Subset(fr_039, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_039_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_040 <- read.FCS("CTRL_Tube_001_040.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_040_gated <- Subset(fr_040, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_040_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_041 <- read.FCS("CTRL_Tube_001_041.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_041_gated <- Subset(fr_041, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_041_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_042 <- read.FCS("CTRL_Tube_001_042.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_042_gated <- Subset(fr_042, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_042_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_043 <- read.FCS("CTRL_Tube_001_043.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_043_gated <- Subset(fr_043, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_043_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_044 <- read.FCS("CTRL_Tube_001_044.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_044_gated <- Subset(fr_044, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_044_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_045 <- read.FCS("CTRL_Tube_001_045.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_045_gated <- Subset(fr_045, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_045_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_046 <- read.FCS("CTRL_Tube_001_046.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_046_gated <- Subset(fr_046, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_046_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_047 <- read.FCS("CTRL_Tube_001_047.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_047_gated <- Subset(fr_047, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_047_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_048 <- read.FCS("CTRL_Tube_001_048.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_048_gated <- Subset(fr_048, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_048_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_049 <- read.FCS("CTRL_Tube_001_049.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_049_gated <- Subset(fr_049, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_049_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_050 <- read.FCS("CTRL_Tube_001_050.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_050_gated <- Subset(fr_050, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_050_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_051 <- read.FCS("CTRL_Tube_001_051.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_051_gated <- Subset(fr_051, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_051_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_052 <- read.FCS("CTRL_Tube_001_052.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_052_gated <- Subset(fr_052, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_052_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_053 <- read.FCS("CTRL_Tube_001_053.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_053_gated <- Subset(fr_053, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_053_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_054 <- read.FCS("CTRL_Tube_001_054.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_054_gated <- Subset(fr_054, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_054_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_055 <- read.FCS("CTRL_Tube_001_055.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_055_gated <- Subset(fr_055, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_055_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_056 <- read.FCS("CTRL_Tube_001_056.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_056_gated <- Subset(fr_056, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_056_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_057 <- read.FCS("CTRL_Tube_001_057.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_057_gated <- Subset(fr_057, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_057_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_058 <- read.FCS("CTRL_Tube_001_058.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_058_gated <- Subset(fr_058, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_058_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_059 <- read.FCS("CTRL_Tube_001_059.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_059_gated <- Subset(fr_059, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_059_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_060 <- read.FCS("CTRL_Tube_001_060.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_060_gated <- Subset(fr_060, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_060_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_061 <- read.FCS("CTRL_Tube_001_061.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_061_gated <- Subset(fr_061, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_061_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_062 <- read.FCS("CTRL_Tube_001_062.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_062_gated <- Subset(fr_062, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_062_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_063 <- read.FCS("CTRL_Tube_001_063.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_063_gated <- Subset(fr_063, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_063_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_064 <- read.FCS("CTRL_Tube_001_064.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_064_gated <- Subset(fr_064, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_064_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_065 <- read.FCS("CTRL_Tube_001_065.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_065_gated <- Subset(fr_065, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_065_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_066 <- read.FCS("CTRL_Tube_001_066.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_066_gated <- Subset(fr_066, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_066_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_067 <- read.FCS("CTRL_Tube_001_067.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_067_gated <- Subset(fr_067, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_067_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_068 <- read.FCS("CTRL_Tube_001_068.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_068_gated <- Subset(fr_068, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_068_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_069 <- read.FCS("CTRL_Tube_001_069.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_069_gated <- Subset(fr_069, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_069_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_070 <- read.FCS("CTRL_Tube_001_070.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_070_gated <- Subset(fr_070, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_070_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_071 <- read.FCS("CTRL_Tube_001_071.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_071_gated <- Subset(fr_071, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_071_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_072 <- read.FCS("CTRL_Tube_001_072.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_072_gated <- Subset(fr_072, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_072_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_073 <- read.FCS("CTRL_Tube_001_073.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_073_gated <- Subset(fr_073, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_073_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_074 <- read.FCS("CTRL_Tube_001_074.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_074_gated <- Subset(fr_074, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_074_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_075 <- read.FCS("CTRL_Tube_001_075.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_075_gated <- Subset(fr_075, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_075_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_076 <- read.FCS("CTRL_Tube_001_076.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_076_gated <- Subset(fr_076, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_076_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_077 <- read.FCS("CTRL_Tube_001_077.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_077_gated <- Subset(fr_077, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_077_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_078 <- read.FCS("CTRL_Tube_001_078.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_078_gated <- Subset(fr_078, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_078_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_079 <- read.FCS("CTRL_Tube_001_079.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_079_gated <- Subset(fr_079, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_079_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_080 <- read.FCS("CTRL_Tube_001_080.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_080_gated <- Subset(fr_080, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_080_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_081 <- read.FCS("CTRL_Tube_001_081.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_081_gated <- Subset(fr_081, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_081_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_082 <- read.FCS("CTRL_Tube_001_082.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_082_gated <- Subset(fr_082, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_082_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_083 <- read.FCS("CTRL_Tube_001_083.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_083_gated <- Subset(fr_083, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_083_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_084 <- read.FCS("CTRL_Tube_001_084.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_084_gated <- Subset(fr_084, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_084_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_085 <- read.FCS("CTRL_Tube_001_085.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_085_gated <- Subset(fr_085, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_085_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_086 <- read.FCS("CTRL_Tube_001_086.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_086_gated <- Subset(fr_086, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_086_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_087 <- read.FCS("CTRL_Tube_001_087.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_087_gated <- Subset(fr_087, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_087_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_088 <- read.FCS("CTRL_Tube_001_088.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_088_gated <- Subset(fr_088, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_088_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_089 <- read.FCS("CTRL_Tube_001_089.fcs", transformation = FALSE)
## Warning: 'read.FCS(.)' is deprecated.
## Use 'read.flowSet(.)' instead.
## See help("Deprecated")
fr_089_gated <- Subset(fr_089, fr_ellipse)
print(paste("Gated events: ", nrow(exprs(fr_089_gated)), sep=""))
## [1] "Gated events: 1000000"

fr_090 <- read.FCS("CTRL_
```

```
autoplot(samples_gat)[[4], m = 'BV710-A', y = 'BV711-A', gs_per_pop_parkescent_gat(c7)], bins = 256]

## Coordinate system already present. Adding new coordinate system, which will replace the existing one.
```

IronoDebris_gate/singlets

CTRL_Tube_001_005.fcs
10.1092%
10.1092%
10.1092%